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review

High density lipoprotein metabolism

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I. INTRODUCTION

Interest in high density lipoproteins was revived in 1975 when Miller and Miller (1) summarized existing data that demonstrated the putative role of HDL as a protective lipoprotein against premature development of atherosclerotic diseases. In that year, the Index Medicus listed 54 publications under the heading "high density lipoproteins" and 82 under the heading "low density lipoproteins"; in 1980, the corresponding numbers were 275 and 139, respectively. It is therefore not surprising that understanding of the HDL system has considerably advanced during the last 8 years. Until the mid-70's, HDL was considered independent of other lipoproteins and was studied mainly as an example of a lipid-protein complex. With the exception of a few studies that dealt with the origin and metabolic activity of HDL, investigations were concerned with the nature of the lipid-protein association. No fewer than three prominent groups published their views of the structure of HDL (2–4) in 1974. Yet, virtually nothing was known about the metabolic behavior of the lipoprotein. That situation has changed since 1975, when intensive investigational efforts produced an outflow of data and concepts on the metabolic activity of the HDL system. The purpose of the present text is to review these newer data and concepts.

II. DEFINITION

HDL particles range in diameter between 70 and 100 Å and in molecular mass between 200 and 400 $\times 10^3$ daltons. Smaller- and larger-sized subclasses have also been identified. Plasma HDL levels are customarily expressed by their cholesterol content. This is unfortunate, since the contribution of cholesterol to the total mass of the lipoprotein is only about 15%. This expression masks the fact that in adult humans the concentra-

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Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; LCAT, lecithin:cholesterol acyltransferase; ABL, abetalipoproteinemia; ACAT, acyl:cholesterol acyltransferase; FCR, fractional catabolic rate; CE, cholesteryl ester; FC, free cholesterol; PC, phosphatidylcholine; LPL, lipoprotein lipase.

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tion of circulating HDL is about 250–500 mg/dl, equivalent to that of LDL. Because more HDL than LDL distributes to extravascular spaces, the total body content of HDL possibly exceeds that of LDL. In younger humans, when LDL levels are relatively low, HDL is the major plasma lipoprotein, and HDL is the major plasma lipoprotein in many animal species. When the number of circulating HDL particles is considered, there is no doubt that HDL is indeed the predominant plasma lipoprotein class: there are 10–20-fold more HDL particles in the human body fluids than the number of all other lipoprotein particles (5).

In spite of their large number in plasma, it is difficult to define HDL as a vehicle for lipid transport. Chylomicrons and VLDL are undoubtedly lipoproteins that carry triglycerides from sites of absorption and synthesis to sites of storage and utilization. LDL, a degradation product of VLDL (6–9), is the major cholesterol-carrying lipoprotein in human plasma. HDL cannot be similarly classified and other considerations must apply to this lipoprotein. HDL is the site of plasma cholesterol esterification and could be defined as the machinery for that important reaction. However, apparently only a small fraction of the HDL is sufficient for that purpose, and there is no indication of absence, or subnormal cholesterol esterification in patients with very low or even complete absence of HDL (10, 11). Another potentially important function of HDL is the so-called "reverse cholesterol transport", i.e., the transport of cholesterol from peripheral tissues to the liver, the main organ of cholesterol utilization. This function, however, reflects the presence of HDL and cannot be used to define the lipoprotein. We prefer to define HDL by its major pathway of origin. As described later in this review (Section IV), HDL can be viewed as a modified product of redundant surface lipids and proteins generated during the process of triglyceride transport. Generation of such redundancies is an obligatory event during chylomicron and VLDL lipolysis. Because surface lipids (free cholesterol and phospholipids) and apolipoproteins are potent detergents that may, and probably are, harmful to body cells, their "packaging" to spherical particles is of biological benefit. Thus, HDL appears to be an essential product of triglyceride transport (as is LDL), and is the major "surface-remnant" of the same process (12–16). This definition of HDL as a product of "surface-remnants" of lipolyzed triglyceride-rich lipoproteins is not only convenient but also compatible with metabolic events discussed in this review.

III. STRUCTURE AND COMPOSITION

A. Lipids and proteins

Circulating HDL consists of spherical particles that conform to the general "core-lipid" structure of lipoproteins (17). In normotriglyceridemic subjects, the major core-lipid is cholesteryl ester. Variable amounts of triglycerides are also present, depending on the presence of lipid transfer activity in plasma and on plasma triglyceride levels. The outer shell (surface) of the HDL contains phospholipids, free cholesterol, and apolipoproteins. Reported values for the lecithin to sphingomyelin molar ratio range between 4:1 to 8:1 (18). In that respect, HDL is similar to chylomicrons and VLDL and is different from LDL, a lipoprotein with relatively high content of sphingomyelin (about 30% of total phospholipid molecules). Assuming that the width of the outer shell is 20 Å, and the diameter of the HDL core is 40 Å (HDL_3) or 60 Å (HDL_2), it is calculated that 80–85% of the HDL volume is in the surface domain. Hence, even spherical HDL is essentially a "surface" lipoprotein.

The distribution of molecules between core and surface in HDL is not categorical. In analogy to triglyceride-rich lipoproteins (19), few cholesteryl ester and triglyceride molecules are apparently present at the surface, while ~40% of the free cholesterol molecules are in the core (20–22). This phenomenon may have important biological implications. Cholesteryl esters and triglycerides present at the surface may represent the molecules that are available for the lipid transfer reactions and the surface may constitute the site of triglyceride hydrolysis by cell-associated lipases; the presence of free cholesterol in the core increases substantially the ability of HDL to accept these molecules from other lipoproteins and from cells.

The nature of protein-lipid associations in HDL has been studied extensively (2–4, 17). In brief, it is believed that helical segments of apolipoproteins interact with phospholipids and possibly other HDL lipids by hydrophobic and ionic forces. Angles between phospholipid head groups, determined by the large radius of curvature of the lipoprotein, enable interdigitation of protein helices between the phospholipid molecules and better interaction of the hydrophobic face of the helix with phospholipid acyl chains and free cholesterol (23) or even with esterified cholesterol molecules (24). It is interesting to note that different apolipoproteins interact differently with the HDL particle. As discussed below (Section V), apoA-I, the major HDL apoprotein, is readily displaced from HDL by other apolipoproteins, whereas apoC molecules are easily added or excluded from the lipoprotein.

B. HDL subclasses

The presence of several discrete HDL classes has been confirmed in many studies. In the studies of Gofman et al. (25), HDL was separated into three subpopulations: HDL_1, HDL_2, and HDL_3. HDL_2 and HDL_3 are undoubtedly the major HDL populations

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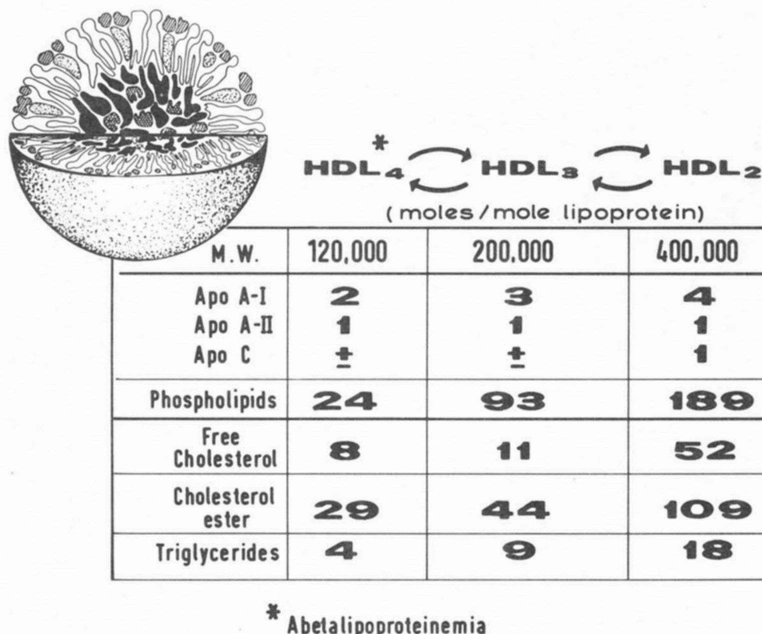
present in the plasma of most, if not all, animal species. The main features of the two are shown in **Fig. 1** and are best illustrated when the content of lipid and protein molecules in each is considered. The core diameter of HDL_2 is about 50% larger than that of HDL_3 (60 and 40 Å, respectively), resulting in a core volume which is 3.5-fold larger. Hence, HDL_2 contains 3–4-fold more cholesteryl ester and triglyceride molecules than HDL_3. The surface area of HDL_2 is also larger than that of HDL_3, but the difference is only 2-fold; the difference in protein content is only 50%. When the molecular weight of the apolipoproteins is taken into consideration, the difference in protein content can be accounted for by one apoA-I and one apoC molecule. Thus, when the number of cholesteryl ester molecules carried in an HDL_2 particle is expressed per one apoA (A-I and A-II) molecule, this ratio is twice as large as that in HDL_3 (15). If the capacity to carry lipids by proteins is regarded as the "efficiency" of a lipoprotein as a vehicle for fat transport, then HDL_2 is twice as "efficient" as HDL_3, at least in that respect. Micropolydispersity has been demonstrated in either class of HDL, and both contain particles that vary somewhat in density, size, and lipid content (26). Yet, HDL_2 and HDL_3 are almost completely separated on density gradients (i.e., rate zonal centrifugation (26, 27)), with a dip between the two. This phenomenon indicates that the two HDL classes represent thermodynamically favorable structures, with only a relatively narrow margin of variation within each class. We have suggested that the addition of one molecule of apoA-I may be responsible for the "quantum" jump between the two (15). Yet, this suggestion is not based on data, and the question of why HDL_2 and HDL_3 are almost totally separable is unanswered.

Several other discrete HDL populations have been described, and their presence in plasma has been confirmed by several investigators. The designation HDL_1 refers to an HDL class that consists of particles rich in apoE and which is lighter and larger than HDL_2. HDL_1 has been identified in human plasma (28–30) and is present in relatively large quantities in rats, a species with very low levels of LDL (31–33). HDL_1 can be separated from other HDL fractions by affinity chromatography on heparin-Sepharose columns leaving behind apoE-free HDL_2 and HDL_3 (28, 29). In the rat, HDL_1 spans the density of 1.04–1.09 g/ml, and is rich in apoE (50–60% of total protein) but also contains relatively large amounts of apoC and small amounts of apoA-I and apoA-IV. The diameter of the lipoprotein is 130–140 Å, and the core volume (and therefore cholesteryl ester content) is about 4-fold larger than that of HDL_2 (33). The predominant cholesteryl ester fatty acid is arachidonic acid, indicating LCAT origin of these molecules. A lipoprotein that is rich in apoE and separates at similar density intervals is consistently found in the plasma of animals fed diets rich in cholesterol (for a review, see reference 34). This lipoprotein is designated HDL_c, and is possibly an analogue of the normally circulating HDL_1.

An HDL population lighter and larger than normal



(moles/mole lipoprotein)	HDL_4*	HDL_3	HDL_2
M.W.	120,000	200,000	400,000
Apo A-I	2	3	4
Apo A-II	1	1	1
Apo C	±	±	1
Phospholipids	24	93	189
Free Cholesterol	8	11	52
Cholesterol ester	29	44	109
Triglycerides	4	9	18

* Abetalipoproteinemia

Fig. 1. Composition of high density lipoprotein subclasses. Data are number of protein and lipid molecules in average particles.

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HDL_2 has been recently identified in the plasma of patients with abetalipoproteinemia (35). Because the main apoproteins of this HDL are apoA-I and apoA-II, we refer to the lipoprotein as ABL-HDL_2. The size and hydrated density of ABL-HDL_2 are similar to those of HDL_1, and the main difference between the two is the apoprotein profile. In that disease we have also identified an HDL population which is denser and smaller than HDL_3, referred to as HDL_4 (35). The particles are spherical, contain mainly apoA-I and apoA-II, and range in diameter between 65 and 70 Å. Not dissimilar tiny HDL populations have also been found in other conditions, e.g., familial LCAT deficiency (36) and cerebrotendinous xanthomatosis (unpublished data). The biological significance of these small-sized HDL populations is unclear.

A totally different approach to separating HDL subpopulations was recently adopted using specific immunoadsorption techniques. In two studies, HDL was separated into populations containing both apoA-I and apoA-II, and populations containing only apoA-I (37, 38). ApoA-I-HDL can be identified in HDL_2 and HDL_3 but seems to prevail in the former. Reported metabolic studies indicated that the plasma residence time of apoA-I in either one of the two types of populations is identical (38).

IV. THE ORIGIN OF HDL

The studies published by Marsh (39, 40) and Hamilton et al. (41) on the nature of lipoproteins that accumulate during isolated rat liver perfusion have demonstrated that HDL is initially formed as a precursor lipoprotein, very different from the circulating particle. The initial particle is a discoidal lipoprotein composed of phospholipids, apolipoproteins, and small amounts of free cholesterol. It has been suggested that the discoidal lipoproteins enter the blood stream and are transformed to the mature spherical particles after interaction with LCAT (41). The view that several metabolic steps are involved with the formation of mature HDL remained a cornerstone in all subsequent thinking on HDL formation. Yet it became clear that additional metabolic pathways contributed to the HDL system.

A. Origin of HDL apolipoproteins

Intestine and liver cells are the major sources of A apoproteins. Although data are mainly available for apoA-I in the rat, the existing information in humans indicates that those data can be extrapolated to the two proteins (A-I and A-II) and that qualitatively similar pathways operate in the two species.

1. The intestine as a source of HDL and HDL apoproteins. The mode of synthesis and secretion of HDL and HDL apoproteins by the intestine has been recently reviewed (42, 43). In intestinal lymph from the fasting and nonfasting rat, apoprotein A-I appears in chylomicrons and high density lipoprotein fractions (44–49). ApoA-I (and apoA-II) were also identified in chylomicrons isolated from human chylous thoracic fluid (50), the urine of humans with chyluria (51), and human thoracic duct lymph (52–55). In rat mesenteric lymph chylomicrons, apoA-I contributes about 50% of total protein (44, 46) and appears to be an important protein constituent of nascent chylomicrons. The presence of apoA-I in intestinal absorptive cells was demonstrated in rats and humans by immunofluorescence techniques (44, 56–59), and apoA-I is secreted by the cultured human intestine (60–62). In studies carried out in humans, transport of apoA-II in lymph has been demonstrated as well (50–54). Windmueller and Wu (48, 49, 63) have shown that at least 50% of the apoA-I in rat plasma originates from the intestine.

ApoA-I content in intestinal absorptive cells and apoA-I transport in the lymph increases considerably during fat absorption (44, 56–58, 64). Yet the basal rates of intestinal apoA-I synthesis and secretion are apparently independent of biliary lipid and are unaffected by biliary diversion (63, 65). It is interesting to note that, in rats with biliary diversion, substantial amounts of newly synthesized apoA-I are secreted via the portal blood circulation rather than with lymph (63).

The amount of apoA-I secreted with chylomicrons (and VLDL) as compared to that with HDL (and other dense lymph fractions) is controversial. Imaizumi et al. (47) reported that 58–74% of the lymph apoA-I appears at $d < 1.006$ g/ml in glucose-fed rats, and these values increase to 82–96% during fat feeding. Other investigators report that only 15–20% of the lymph apoA-I is associated with intestinal VLDL in fasting rats (44, 51, 54); fat feeding increases these values to 40–50% (44). This discrepancy is not surprising in view of the difficulties of assessing the amounts of newly synthesized apoA-I that appear with different lipoproteins. One difficulty is filtration of HDL (and of proteins) from plasma to lymph (47, 65). In a study by Anderson et al. (54), the measured lymphatic transport of apoA-I exceeded that of predicted apoA-I production by as much as 2.6-fold. Another difficulty is the mode of secretion of intestinal lipoproteins and the tortuous route that lipoproteins traverse before entering lymph. Lipoproteins assembled in intestinal absorptive cells are secreted through the lateral walls of the cells, cross the basal membrane, and traverse the lamina propria before entering lymph lacteals (66). Along this road the lipoproteins may interact with many different cells, including fibroblasts, smooth muscle cells, macrophages, and oth-

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ers. Interactions of chylomicrons with such cells or their secretory products could result in displacement of apoA-I from the chylomicrons. Conversely, association of secreted unbound apoproteins with the lipoprotein particle may take place. Because of all these reservations, the finding that as much as 80% of lymph apoA-I is found in fractions denser than chylomicrons should be interpreted with care.

The nature of HDL particles secreted by the intestine is unclear. Both discoidal and spherical particles were identified in rat mesenteric duct lymph (42–45). Discoidal particles contribute about 50% of total lymph HDL in fasting rats and 35% in fed rats. The relative number of discoidal particles increases when LCAT inhibitor is added, and it has been suggested that the discoidal HDL represents "nascent" lipoproteins, while the spherical particles represent HDL filtered from plasma. In human thoracic duct lymph and in HDL isolated from the urine of humans with chyluria, however, discoidal lipoproteins were not identified (51, 54). Human thoracic duct lymph contains a predominance of HDL_2 particles (compared to the plasma HDL of the same subjects) (54). As well, discoidal HDL particles were not yet identified in the enterocyte. More recently, Forester et al. (67) have suggested that intestinal cells secrete spherical HDL particles of small diameter. That suggestion agrees with conclusions derived from observations in patients with abetalipoproteinemia, that the intestine secretes a small-sized and dense HDL population (35). As discussed below, the finding of discoidal HDL in lymph cannot be considered as a definite proof that such particles are indeed primary secretion products of the enterocyte; it is, however, possible that spherical HDL, containing core-lipids, are secreted by intestinal absorptive cells. It is noteworthy that intestinal cells contain a relatively high activity of the enzyme system acyl:cholesterol acyl transferase (ACAT) (68, 69), and the intestine is the major source of plasma cholesteryl esters in patients with LCAT deficiency (70).

2. The liver as a source of HDL and HDL apoproteins. Biosynthesis and secretion of lipoproteins and apolipoproteins, including HDL, by the rat liver was extensively studied in the late 1950's and during the 1960's (71). Later, individual HDL apoproteins were identified (39, 72, 73), and characterized (40, 41, 74). Marsh (40) reported his investigations on the nature of newly synthesized HDL apoproteins identified in non-recirculating rat liver perfusates in 1976. In livers from normal rats, apoE, apoA-I, and apoC comprise more than 95% of total HDL proteins. In contrast to serum HDL, perfusate HDL contains more apoE than apoA-I (weight ratio of 1.7). To eliminate contamination of HDL with VLDL, the experiment was repeated in livers from rats treated with orotic acid. In these perfusates, VLDL release is 15% of normal, and HDL, 40%. The HDL contained an even higher ratio of apoE to apoA-I, 5.0. ApoC contributed one-third of total HDL proteins with either preparation. Addition of ^{14}C-labeled amino acids revealed that the major labeled apoprotein was apoE (64% of radioactivity) followed by apoC (16% of radioactivity). ApoA-I contained only 3% of total radioactivity (specific activity less than one-tenth that of apoE, and about one-fourth that of apoC). Hamilton et al. (41) also found that apoE is the major perfusate HDL protein, especially when the LCAT reaction is inhibited during the perfusion period. Rates of apoE secretion increase with time of perfusion whereas secretion of apoA-I decreases, or even levels off (74). In HDL (separated as HDL_2 and HDL_3), apoE to apoA-I weight ratios were as high as 10.4 (in the presence of DTNB). Minimal amounts of apoA-I were found in VLDL. Stimulation of apoE and apoA-I secretion was observed in rats with nephrosis (75). In livers from such rats, apoE secretion increased by a factor of 1.8, and apoA-I by 8.4. The perfusate HDL from nephrotic rats contained more cholesteryl esters (9.0% of lipid mass compared to 4.1% in controls) and was markedly enriched with apoA-I, 52.3% of total protein (compared to 15.7% in control rats). The incorporation of labeled amino acids into apoA-I also increased markedly in livers from nephrotic rats, by 441% (compared to 38% for apoE).

ApoA-I has been demonstrated in human hepatocytes (59), is secreted by perfused livers of several different animals (76, 77), and by rat hepatocytes in culture (78, 79). ApoA-I secretion by hepatocytes is unaffected by adding fatty acids to the medium (79), but increases in liver perfusates from fed pigs (76). Cholesterol feeding doubles apoA-I production in the guinea pig (77), but apoA-I production does not change when rats are made hypothyroid or hypothyroid-hypercholesterolemic (78). Synthesis and secretion of apoA-I by other tissues have been reported in avians (80, 81). Yet, in mammals, mRNA for apoA-I is found only in the liver and gastrointestinal tract (82).

B. Origin of lipids and formation of HDL precursors

HDL precursors are defined here as apoprotein-phospholipid-free cholesterol complexes that are formed in vivo and can be transformed to spherical particles by the LCAT reaction. This definition does not specify the nature of apoproteins associated with the HDL precursors, although apoA-I and apoA-II constitute the major proteins of the circulating lipoprotein. Three processes must be considered as sources of HDL precursors: *a*) direct secretion of discoidal high density structures from hepatic and intestinal cells ("nascent" HDL particles); *b*) lipid and protein constituents released from lipolyzed

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triglyceride-rich lipoproteins ("surface remnants"); and *c*) phospholipid-apoprotein associations, not dissimilar to those formed in vitro.

The discoidal structures identified in liver perfusates and intestinal lymph, were initially suggested to be the major, if not the only source of nascent HDL. That view, however, was soon challenged by us (12, 14). Several considerations led us to doubt the view that the discoidal high density particles are true secretory products of cells. The first was the inability to identify such structures along intracellular secretory pathways (41, 42, 74), although other lipoproteins are abundant in intracellular organelles and can readily be isolated from the Golgi apparatus. Because of their relatively small size, it was expected to identify "nascent" HDL inside cells (especially in Golgi cisternae or secretory vesicles) even if the total amount of protein found in the lymph or perfusate is not very large. Could such discoidal structures originate from the surface layer of secreted VLDL and chylomicrons? The liver is a major source of a triglyceride lipase (83–85) that is highly active under the conditions of the perfusion procedure, i.e., using medium devoid of plasma or plasma lipoproteins. [Plasma fractions, and in particular HDL, are known inhibitors (competitive inhibitors?) of the hepatic lipase activity against triglyceride-rich lipoproteins (83, 86).] Indeed, we showed that VLDL triglycerides are readily taken up by the liver during recirculating perfusion, and that the release of the lipase by heparin results in prompt hydrolysis of the VLDL triglycerides in the perfusate and uptake of the released fatty acids by the liver (86). Addition of plasma or of lipoproteins to the perfusion medium prevented these processes. As to the intestine, we postulated that chylomicrons could interact with lipases when they traverse the lamina propria. A potential source is lipoprotein lipase secreted by macrophages (87, 88). Comparison of the apoproteins associated with the hepatic and intestinal discoidal structures support our arguments. ApoE (and not apoA-I) is the major apoprotein found with the hepatic particles, whereas apoA-I is predominant in the intestinal particles. These are the major apoproteins found with the triglyceride-rich lipoproteins secreted from the two organs, respectively. Finally, in the studies demonstrating the presence of discoidal lipoproteins in liver perfusates or intestinal lymph, it was not possible to rule out the possibility that the cells secrete free apoproteins that become associated with phospholipids at a later stage of the secretory process or even after secretion occurred. With the former possibility, hepatic or intestinal cell membrane would serve as a phospholipid source; in the latter case, phospholipids would be derived from cells and secreted triglyceride-rich lipoproteins.

The second potential source of HDL precursors is the surface coat of lipolyzed triglyceride-rich lipoproteins. Transfer of VLDL and chylomicron constituents to HDL during the course of lipolysis was already demonstrated during the 1950's (89) and confirmed in many subsequent studies. However, it was not until the mid 70's that the full significance of these observations became apparent. Our earlier studies focused on the composition and structure of VLDL and post-lipolysis VLDL (7, 90, 91). These studies demonstrated unequivocally that when VLDL is lipolyzed in vitro, as much as 80% of the phospholipids and free cholesterol, and practically all of the C apoproteins, are excluded from the lipoprotein. In subsequent studies, we specifically studied the form by which surface constituents leave the VLDL. The study included in vitro lipolysis experiments (92–96) and an investigation of VLDL-lipolysis in a recirculating isolated rat heart perfusion system (97). With all systems, lipolysis was carried out in media devoid of plasma lipoproteins. Albumin, however, had to be included, and in some instances the albumin used was contaminated with bovine apoA-I (98) and with phospholipids (92). Regardless of the system used, the experiments showed consistently that phospholipid, free cholesterol, and apoC molecules are displaced from the surface of lipolyzed VLDL and can be recovered with high density fractions. After isolation, the high density buffer fraction contained an abundance of discoidal structures. [More recently, we observed heterogeneity of "surface remnants", and some vesicular and spherical structures are identifiable as well (unpublished data).] These findings were subsequently confirmed by other investigators (99–101), and essentially identical data were reported for human thoracic duct chylomicrons (102). Thus, the data strongly suggest that constituents originating from the surface coat of lipolyzed triglyceride-rich lipoproteins constitute the major, if not the only, source of HDL precursors (12–16). Yet, several different mechanisms may be involved with formation of HDL precursors. One mechanism is release, or exclusion of fragments of surface redundancies from the shrinking chylomicron or VLDL particles. Tall and Small (13) suggested that apoA-I is necessary for fragmentation of the surface coat of lipolyzed triglyceride-rich lipoproteins. This, however, has not been demonstrated experimentally and we have identified discoidal or vesicular high density structures even when lipolysis was carried out in the absence of apoA-I (95). Other mechanisms may operate and even predominate. For example, it is possible that lipid and apoprotein molecules are excluded individually from the surface of lipolyzed lipoproteins and that the discoidal (or spherical) structures are formed in the water phase.

The third pathway by which HDL precursors can be formed is through apoprotein-phospholipid associations.

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According to this view, shortly after an apolipoprotein becomes dissociated, it forms a complex with phospholipids (mainly phosphatidylcholine) derived from cells or lipoproteins. This pathway is shown schematically in **Fig. 2.** The apoA-I pool represents the amount of protein available for HDL formation. This pool is derived from two sources: secretion of *free* apoproteins by cells that synthesize and secrete apolipoproteins, and apoproteins released from lipolyzed triglyceride-rich lipoproteins. ApoA-I-phospholipid complexes can form rapidly if phospholipids are available. The figure suggests that phospholipids are derived from either cell membranes and intact lipoproteins or the surface coat of lipolyzed triglyceride-rich lipoproteins. True HDL precursors are formed when free cholesterol molecules are added to the apoprotein-phospholipid complex. Again, cells, intact lipoproteins, and lipolyzed lipoproteins are the sources of free cholesterol.

Several features of the scheme are noteworthy. *Firstly,* the scheme is compatible with the previous two sources of the precursors. Formation of "nascent" HDL can be viewed as a similar metabolic sequence, occurring inside cells: apoproteins that are not part of VLDL or chylomicrons become associated with phospholipids along their secretory path, and appear in intercellular spaces as protein-phospholipid complexes. Because such complexes have not been identified in the cells, the association must occur late in the secretory path, possibly even when the proteins cross the plasma membranes. With regard to the second source of HDL precursors, it has been mentioned above that apoprotein-phospholipid-free cholesterol complexes may leave the surface of lipolyzed lipoproteins as "surface remnants". In these two instances, the "nascent" complexes or the "surface remnants" contribute directly to the HDL precursor pool. Conceptually, the three routes are indistinguishable and represent pathways that accommodate unassociated apoproteins. *Secondly,* the scheme predicts that formation of HDL depends on two processes: secretion of free apoproteins and lipolysis of triglyceride-rich lipoproteins. That seems necessary in view of the very strong relationship that exists between lipoprotein lipase activities and HDL levels (103–106) and the presence of HDL in plasma even when lipolysis is totally absent (e.g., abetalipoproteinemia) or is extremely limited (e.g., lipoprotein lipase or apoC-II deficiencies). *Thirdly,* although apoA-I is the only protein shown in the scheme, other apoproteins can substitute for apoA-I, at least in part. Thus, apoC, apoE, or apoA-II can initially be part of the apoprotein-phospholipid complex. *Finally,* the scheme

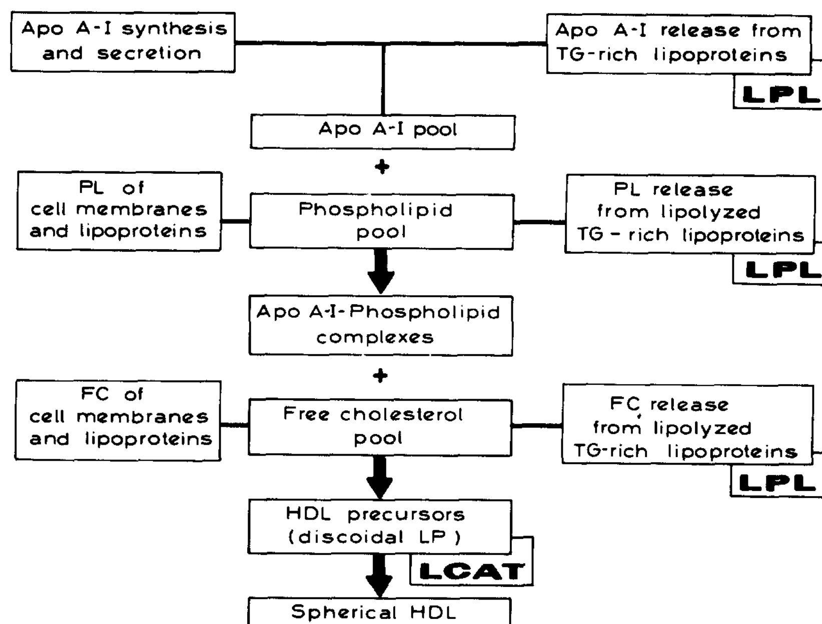

Fig. 2. Schematic representation of HDL formation. It is suggested that apoproteins, phospholipid, and free cholesterol become associated in body fluids and form HDL precursors (discoidal lipoproteins). The sources of apoproteins are synthetic sites (predominantly liver and intestine) and the lipolysis process. Phospholipids and free cholesterol molecules are derived from the surface coats as a result of lipolysis of triglyceride-rich lipoproteins and from membrane and lipoprotein lipids. "Nascent HDL" and "surface remnants" contribute directly to the HDL precursor pool (see text). Spherical HDL is formed when sufficient cholesteryl esters are generated via LCAT.

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predicts that formation of HDL precursors necessitates *supply of both apoproteins and phospholipids.* Under physiological conditions, phospholipid supply apparently is not limited. In many experimental models (e.g., lipolysis) however, hydrolysis of phospholipids (one action of lipoprotein and hepatic lipases, refs. 86, 93, 95, 97, 107–110) may obscure processes that during normal physiology are followed by formation of HDL precursors. We have shown that, in the presence of excess lipase, as much as 70% of the VLDL phosphatidylcholine can be hydrolyzed in vitro (95). Of interest, when VLDL triglycerides are hydrolyzed in vitro by a bacterial lipase devoid of phospholipid hydrolase activity, the amount of "surface remnants" recovered in the test tube is 2- to 4-fold higher as compared to systems with the same degree of triglyceride hydrolysis achieved by excessive amounts of lipoprotein lipase (111).

C. Formation of spherical HDL

Transformation of HDL precursors to spherical HDL is dependent on LCAT activity. Hamilton et al. (41) have demonstrated that complexes isolated from rat liver perfusates are indeed substrates for LCAT, and suggested that the cholesteryl esters formed at the surface of the complexes are displaced to the hydrophobic hydrocarbon domain of the phospholipid bilayer. Yet rearrangement of the complex seems mandatory. Hamilton et al. (41) pointed out that their complexes contain surplus phospholipids, some of which must be removed during the disc → sphere transformation. As well, apoproteins other than A-I that are present in the complexes must be replaced by apoA-I. This seems obligatory inasmuch as in diseases where apoA-I is absent (10, 11), HDL is not found in plasma. In these conditions, LCAT is active, and cholesteryl esters are present in both VLDL and LDL, although in reduced amounts. Tangier disease provides a unique opportunity to test the consequences of limited supply of apoA-I on HDL formation (112–115). In this disease, "surface remnants" are readily demonstrable after a fatty meal, especially in the patient who underwent splenectomy (114, 115). HDL levels, however, are exceedingly low, and disc → sphere transformations are severely limited in spite of apparently normal synthesis and secretion of all other apoproteins. Small amounts of apoA-II containing spherical HDL are found in the patients' plasma (114), but even apoA-II cannot effectively replace apoA-I. The "surface remnants" and HDL precursors are rapidly cleared from the plasma, predominantly by cells in lymphatic tissues (116).

The classic example of the consequences of the absence of a disc → sphere transformation is familial LCAT deficiency (70). The patients' plasma contains abundant amounts of discoidal and vesicular structures (117–119) and, as in Tangier disease, "surface remnants" accumulate after a fatty meal (120). Both apoA-I and apoA-II have been identified in several HDL populations, while apoE was identified in others (36, 70, 121). The patients' HDL contains about 10–15% of the normal amounts of apoA-I and apoA-II but almost twice as much apoE (36, 122, 123). In whole plasma, the amount of apoA-I is about 40% of normal, but apoA-II levels are low, 10–30% (36, 119, 122, 123). After centrifugation, 70–80% of the apoA-I is found in the plasma fraction of d > 1.25 g/ml, compared with only 3–5% in normal subjects (120). More apoA-I is found with HDL when the patients' plasma is incubated with LCAT, while apoE moves to VLDL (120). In another study, disc → sphere transformations were demonstrated for the apoA-I-containing structure, but not for the apoE particles (124). While it is extremely difficult to interpret all of the data in LCAT deficiency, several conclusions can be drawn. Unlike Tangier disease, LCAT-deficient plasma contains an abundance of HDL precursors, and transformation to spheres is readily demonstrable when LCAT is added (120, 124). The differences between the two conditions are unclear, but possibly reflect the presence of apoA-I in LCAT deficiency, and its nearly total absence in Tangier disease. Another difference is the presence of multiple HDL precursors in LCAT deficiency and their absence in Tangier disease. Is it possible that the presence of LCAT but absence of normal apoA-I causes accelerated catabolism of HDL precursors or of spherical HDL containing abnormal apoprotein composition? These and other questions indicate that disc → sphere transformation may reflect a process more complicated than cholesterol esterification and displacement of cholesteryl ester molecules to the center of the phospholipid bilayer.

D. Genetic control of HDL apoprotein synthesis

The mode of synthesis and secretion of HDL apoproteins A-I and A-II and the genetic control of this process have been clarified over the last 2 years (125).

ApoA-I can be separated by two-dimensional electrophoresis techniques into several (six) isoproteins with the same apparent molecular weight but different isoelectric points (62). The main circulating apoA-I forms are A-I_4 (79 ± 7% of total apoA-I) and A-I_5 and A-I_6 (19 ± 6%); A-I_2 and A-I_3 constitute less than 2% (126). Examination of apoA-I secreted by either hepatic or intestinal tissues in culture (62, 127–133) or by human-derived hepatoma cells (134) revealed that the newly formed protein is different from the circulating protein. ApoA-I_2 and A-I_3 are the major secreted apoproteins, 78 ± 5 and 21 ± 5% of total protein, respectively, while isoforms A-I_4 and A-I_5 constitute less than 1% (126). Hence, it appears that apoA-I is secreted as a proprotein

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($A\text{-}I_2$) that is converted to a mature protein ($A\text{-}I_4$) in the plasma compartment. Sequence analysis of pro-apoA-I revealed that it contains a six-amino-acid N-terminal extension (132, 133, 135–137). Cleavage of this hexapeptide must occur after secretion by a plasma protease (138). Defective conversion of pro-apoA-I to the mature protein has been suggested as one explanation for the abnormal metabolism of apoA-I in Tangier disease (126, 136, 137, 139).

The primary translation product of human apoA-I mRNA contains an additional peptide of 18 amino acids (130, 133, 135, 136, 140). This peptide is cleaved intracellularly by signal peptidase of the endoplasmic reticulum. The resulting protein is pro-apoA-I ($A\text{-}I_2$) described above (134). The gene coding for human (141–144) and mouse (82) apoA-I has recently been isolated and characterized. The gene is approximately 2.0 kb long, contains three intervening sequences, and is localized in human chromosome 11 (125, 142). ApoA-I gene is located about 2.6 kb upstream of the human apoC-III gene and a DNA inserted at this region (145–147) explains the syndrome of apoA-I and apoC-III absence (11). The apoA-I gene contains six highly homologous tandemly repeated 66 bp regions indicating internal gene duplication (125, 142). ApoA-I mRNA concentration in the liver and intestine of the mouse is similar (82), but is undetectable in other tissues of the mouse (82) or rat (148).

Apparently apoA-II is also translated as a preproprotein (133, 149) and is secreted as a proprotein extended by an N-terminal hexapeptide (133). The signal sequence consists of 17 amino acids (133). In the second study however (149), the signal peptide was reported to contain 18 amino acids, while the propeptide comprised five residues.

V. INTRAVASCULAR DYNAMICS OF THE HDL SYSTEM

High density lipoproteins serve in plasma as a reservoir for lipids and apoproteins. The HDL particle, moreover, seems to interact with most, if not all, enzymes and lipid transfer proteins present in plasma and in close proximity to the plasma compartment. These include the two lipases (lipoprotein lipase and the hepatic triglyceride hydrolase), the LCAT system, and the lipid (cholesteryl ester/triglyceride/phospholipid) transfer proteins. For the sake of simplicity, the impact and potential metabolic significance of these processes on HDL are discussed in terms of species of HDL particles. However, it should be clear that, in most instances, groups of particles participate, or are affected simultaneously, by the same metabolic process. The impact of these processes on HDL subpopulation distribution is described in the next section.

A. HDL phospholipids

The available information on HDL phospholipids concerns mainly the phosphatidylcholine (PC) moiety. Four potential sources contribute PC molecules to HDL: "nascent HDL", nascent chylomicrons, the surface coat of lipolyzed triglyceride-rich lipoproteins, and cell membranes (**Fig. 3**). Contribution of phospholipids with nascent HDL occurs only with secreted particles, such as spherical or discoidal HDL of intestinal and hepatic origin. The second source is nascent chylomicrons. Newly secreted chylomicrons (isolated from the lymph) contain surplus phospholipids, which are rapidly transferred to HDL even before any metabolic event takes place (46). The contribution of this pathway to the overall supply of PC to HDL is unknown, but is possibly quite significant. Lipolysis of triglyceride-rich lipoproteins is apparently the major source of HDL-phospholipids. It has been repeatedly shown that HDL becomes enriched with phospholipids during the clearance of alimentary chylomicronemia in humans (150) or after the injection of mesenteric lymph chylomicrons to rats (151, 152). This process is even more pronounced in hepatectomized rats (152). VLDL lipolysis also contributes phospholipids to HDL. The contribution of PC to total chylomicron mass is about 4–5%, and in VLDL, 10–15%. Assuming that 50–80% of the PC molecules are potentially transferable to HDL, lipolysis alone may provide the HDL with 4–8 g (5–10 mmol) of phosphatidylcholine in 1 day (calculated for an adult human). That amount is equivalent to total HDL phospholipid mass and to the amount of PC used daily by the LCAT reaction. Lipolysis, therefore, may provide the HDL system with all the needed PC molecules. The role that phospholipid transfer (exchange) proteins present in human (153, 154) and rat (155, 156) plasma may play in PC transport has not been fully evaluated. Facilitation of transfer was reported between PC vesicles and HDL (157), and more recently, from lipolyzed VLDL to HDL (158). PC molecules may be transferred from cell membranes to HDL. Apoprotein or lipoprotein interaction with the membranes is most probably the major mechanism by which cells can supply phospholipids to the HDL pool. Whether cells do supply HDL with significant amounts of phospholipids, however, is doubtful, as the phospholipid composition of cell membranes is very different from that of HDL.

Once PC molecules are in HDL, they are either consumed or exchanged with the same molecules in other lipoproteins or cell membranes. Phospholipid exchange occurs between HDL and cell membranes and between HDL and other lipoproteins (159, 160). The

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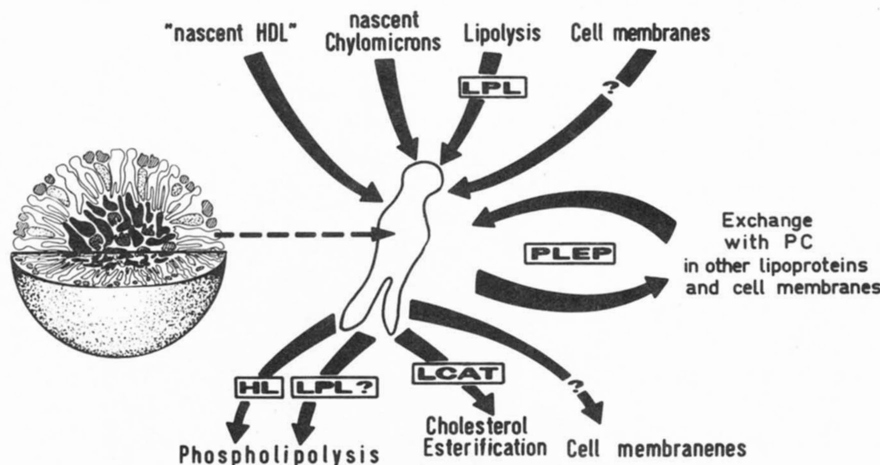

Fig. 3. Intravascular dynamics of HDL phosphatidylcholine (PC). Lipolysis appears to be the major source of HDL-PC (see text). PC also enters HDL with "nascent" particles and is derived from the surface coat of nascent chylomicrons. HDL-PC exchanges with PC in other lipoproteins and cell membranes, a reaction that is facilitated by phospholipid exchange proteins (PLEP). PC is hydrolyzed by lipases and is consumed by the LCAT reaction. Whether there is net movement of PC molecules between HDL and cell membranes is not clear. The lifetime of PC molecules in HDL is less than 1 day.

latter has been shown to be greatly facilitated by a plasma phospholipid exchange protein (154–156). In our study (155), biosynthetically labeled rat plasma VLDL served as the donor of labeled phospholipids. In the presence of plasma, an almost complete exchange of phospholipids between VLDL and HDL (equal specific activity of PC) was achieved after 60 min of incubation. The reaction, however, did not result in net movement of molecules in either direction. In analogy with other PC exchange reactions (161, 162), it is expected that such proteins may also facilitate exchange with cell membranes. Under normal conditions, this exchange also does not cause net movement of PC molecules between the lipoproteins and the cells. Yet, when phospholipids are consumed at a rapid rate by cells (e.g., rapidly growing tissues) or lipoproteins (e.g., LCAT activity), PC molecules may move in either direction.

HDL phospholipids participate in metabolic events and are continuously consumed. Most important is the LCAT reaction which forms cholesteryl esters from PC and free cholesterol molecules. About 5–10 mmol of cholesteryl esters are formed in a day in adult humans by this reaction (70). Hence, the reaction consumes equimolar amounts of PC, i.e., 5–10 mmol or 4–8 g per day. It should be realized that this amount is equivalent to total circulating HDL-PC mass, assuming that 1 liter of plasma contains about 0.5–1.0 g of HDL-PC and that about 60% of the HDL is present in the plasma compartment. HDL-PC, moreover, is available to other enzymes with phospholipase activity, mainly the lipoprotein lipase (108, 109) and the hepatic lipase (163–166) systems. The exact contribution of phospholipid lipolysis to total consumption of HDL-PC is not known.

The considerations presented above demonstrate the dynamic nature of the HDL-phospholipid system. PC molecules continuously enter HDL particles and are rapidly exchanged or consumed. At equilibrium, when the rates of consumption of HDL-PC are considerably shorter than a day, these molecules turn over in HDL at least five times faster than apoproteins.

B. HDL free cholesterol

The dynamics of free cholesterol (FC) in HDL are similar to those of PC (**Fig. 4**). As for PC, the sources of free cholesterol are nascent particles, the surface coat of lipolyzed triglyceride-rich lipoproteins and cell membranes. FC molecules exchange rapidly between lipoproteins (167). At 37°C, more than 90% of ^{14}C-labeled FC in HDL_3 is transferred to LDL in a first order process and $t_{1/2}$ of 2.9 min. For comparison, the $t_{1/2}$ of PC exchange is 5.1 ± 1 hr. FC exchange occurs by diffusion through the aqueous phase (167). Experiments carried out with liposomes have documented rapid distribution of free cholesterol from structures with a high FC/PC molar ratio, to structures with low ratio (168–170). This movement of FC between particles also occurs through the aqueous phase (168, 170). Once in HDL, FC molecules exchange with others in other lipoproteins and cell membranes (171). Under suitable conditions, free

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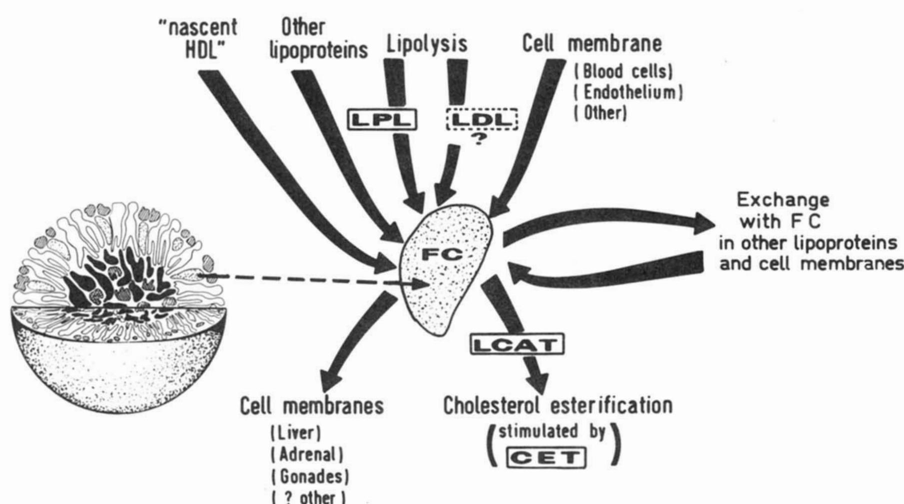

Fig. 4. Intravascular dynamics of HDL free cholesterol (FC). The main sources of FC molecules are the surface coat of intact lipoproteins and lipolyzed triglyceride-rich lipoproteins, cell membranes, and "nascent" particles. With lipolysis, some free cholesterol molecules become first associated with LDL but thereafter are utilized in HDL. HDL-FC exchanges with FC in other lipoproteins and cell membranes, and is utilized by the LCAT reaction (esterification stimulated by cholesteryl ester transfer (CET)). Finally, FC molecules also move from HDL to cells. The lifetime of FC molecules in HDL is a few hours.

cholesterol may move out of HDL to FC-poor structures, but in biological systems this probably occurs infrequently. HDL-FC, like PC, is utilized in the LCAT reaction.

Recently, we have attempted to quantitate the amounts of FC transferred from lipolyzed triglyceride-rich lipoproteins to HDL (172). Specifically, we wished to determine amounts of free cholesterol that are transferred during lipolysis to cell membranes, as compared to the amount transferred to HDL and other lipoproteins. The experiments were conducted in vitro and in the isolated perfused rat heart. Rat plasma VLDL containing biosynthetically labeled [^{3}H]cholesterol was used as the triglyceride-rich lipoprotein, and human blood cells (RBC's, platelets, and WBC's) or the rat heart were potential acceptors for cholesterol. Exchange of free cholesterol between the VLDL and the cells was relatively slow, about 10 hr. With induction of lipolysis, it was found that none of the cholesterol generated during lipolysis was transferred to any of the cells tested. HDL, in contrast, served as an excellent acceptor for this cholesterol, either in the presence or absence of cells. *Thus, it seems that all of the free cholesterol generated during lipolysis remains in the lipoprotein system, and none is taken up by cells.* Of interest was the observation that LDL also serves as acceptor for lipolysis-generated FC. LDL in this cycle, however, seems to play a transient role as we were able to show that FC in both VLDL and LDL is consumed by HDL when the LCAT reaction is allowed to progress (unpublished results). A similar observation was previously reported by Fielding and Fielding (173). We therefore concluded that VLDL and LDL are important sources of FC for HDL, and that the process is accelerated by lipolysis. Whether phospholipids behave similarly has not been determined.

Another quantitative difference between PC and FC is the ease with which FC in cell membranes is transported to HDL. Apparently, when the LCAT reaction is active and HDL-FC becomes esterified, FC molecules move from the membranes to the HDL. This phenomenon is an essential part of the "reverse cholesterol transport" process and is described in detail in Section VIII.

HDL-FC is consumed by the LCAT reaction, and may be used by cells. It has already been mentioned that 5–10 mmol of CE are formed in a day by the LCAT reaction. The whole HDL system (intra- and extravascular) contains at most 1 mmol of FC. *Hence, in a day, the LCAT reaction uses five to ten times more FC than is actually present in HDL.* In other words, all of the HDL-FC is replaced by new molecules within 2–5 hr. In the circulation, FC molecules consumed by the LCAT reaction are replaced by molecules coming from other sources. Such sources are other lipoproteins and cell membranes. Indeed, when plasma is incubated in vitro and the LCAT reaction is allowed to progress, both

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VLDL and LDL become depleted of FC molecules (173). We have studied the kinetics of this process by using [^{14}C]cholesterol-labeled rat plasma supplemented with human VLDL (unpublished results). During the first 6–12 hr of the incubation, utilization of FC was evident only in VLDL and LDL, while the amount of FC in HDL was not changed. Only when 30–50% of the VLDL-FC and LDL-FC was esterified, did the amount of HDL-FC begin to decline. Because the rat is an animal devoid of the core-lipid transfer reaction, we could also show that more than 90% of the newly formed ^{14}C-labeled CE molecules remained in HDL. Hence, the CE molecules were indeed formed in HDL, and the source of FC was transfer of molecules from VLDL and LDL to HDL.

Movement of FC molecules from HDL to cells occurs under special conditions. For example, in LCAT deficiency cholesteryl esters are not formed, and cell membranes (e.g., erythrocytes) are enriched with FC presumably originating from the abnormal lipoproteins present in the plasma (174). Of interest, normal HDL enriched with FC also donates cholesterol to cells in culture (175). Other examples are tissues that utilize large amounts of cholesterol for metabolic activities, i.e., liver, adrenal, and gonads. In the liver, HDL-FC may actually be a major precursor for bile acids (176).

In summary, like PC, the free cholesterol moiety of HDL must turn over at a very fast rate. This rate is probably several-fold faster than that of PC, and 20- to 30-fold faster than apoproteins. HDL, therefore, seems to play a central role in plasma FC metabolism, as suggested by Schwartz et al. (177, 178).

C. HDL cholesteryl esters and triglycerides

The metabolic behavior of the two core lipid moieties of HDL is dependent on three processes: cholesterol esterification via the LCAT reaction, the plasma lipid transfer reaction, and the activity of lipases. The mechanism of the LCAT reaction has been summarized recently. It is appropriate, however, to describe briefly the second reaction, i.e., the lipid transfer reaction. Nichols and Smith (179) were the first to show that, during in vitro incubation of human plasma, triglycerides are transferred from VLDL to LDL and HDL, and cholesteryl ester molecules are transferred in the opposite direction. Several years later, the reaction was demonstrated with isolated lipoproteins (180). In 1975, proteins that catalyze the reaction were found in rabbit lipoprotein-free plasma (d > 1.21 g/ml) (181). Purification of core-lipid transfer proteins was reported by several investigators (156, 182–188). Fielding and his associates (182, 189) suggested an identity between the lipid transfer protein and apoD. This suggestion, however, was not corroborated in other studies, and it is currently believed that the lipid transfer protein is an acidic glycoprotein with a molecular weight of about 61,000 (185–187). Whenever studied, cholesteryl ester, triglyceride, and phospholipid transfer activities are co-purified (156, 184, 186).

In plasma, the transfer protein is associated with HDL, mainly HDL_3, and almost no activity is found with lower density lipoproteins (190, 191). A plasma inhibitor of the transfer activity has been described (192). Cholesteryl ester and triglyceride transfer reactions were demonstrated in many in vitro incubation systems: whole plasma, plasma fractions, and isolated lipoprotein (33, 156, 173, 179, 180, 193–205). While the mechanisms of the reaction are poorly understood, several features of the transfer process have been established. Transfer activity is present in some animal species, including humans and rabbits, but is absent in others such as rats, pigs, and dogs. The bidirectional transfer of triglycerides and cholesteryl esters is always related to the amount of transfer protein, the time of exposure between donor and acceptor particles, and the relative mass of the two. At least two distinct processes can be differentiated: exchange (replacement of a molecule in one lipoprotein by the same molecule present in another lipoprotein) and unidirectional transfer of molecules from one lipoprotein to another. This second reaction is responsible for the changed core-lipid composition that occurs during the exposure of two lipoproteins of different core composition to transfer activity. It has been suggested that the mechanism of both reactions (exchange and unidirectional transfer) depends on interaction of the protein with molecules present at the surface coat of the lipoproteins and is proportional to their surface area (206). The magnitude of exchange and transfer, however, is determined by the relative proportion of cholesteryl ester and triglyceride molecules in different lipoproteins (206). For example, incubation of LDL and HDL, two cholesteryl ester-rich lipoproteins, results mainly in exchange; incubation of LDL or HDL with triglyceride-rich lipoproteins, in contrast, results in altered core-lipid composition of the two lipoproteins (204, 206, 207).

Of particular relevance for understanding HDL core-lipid physiology is the interaction between the LCAT and the core-lipid transfer systems. Removal of cholesteryl esters from HDL has been reported to accelerate the rates of the LCAT reaction, possibly by relieving the HDL of product inhibition (173, 208). It has even been suggested that both the LCAT protein and the core-lipid transfer protein are present together on a specialized HDL subpopulation fraction (189). Newly formed (LCAT-derived) cholesteryl ester molecules can apparently be distributed to lipoproteins by two pathways: a direct pathway, by which the molecules are

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directly transferred to other lipoproteins, and an indirect pathway, by which they are first assimilated in existing HDL particles and are transferred from the HDL to lower density lipoproteins (200, 201, 207). Once the newly formed LCAT-derived cholesteryl esters are in the HDL, however, they seem to be totally miscible with the existing (also LCAT-derived) esters (198). LCAT activity has been reported to increase the amount of cholesteryl ester molecules that are transferred to lower density lipoproteins (173, 198, 206, 207). In human plasma, as much as 80% or even more of the LCAT-derived cholesteryl esters seem to end in lower density lipoproteins (209).

With that brief background, it is now possible to discuss the dynamics of the HDL core-lipid system (**Fig. 5**). The major source of HDL cholesteryl esters is cholesterol esterification by the LCAT reaction. This is evidenced from the cholesteryl ester fatty acid profiles; in both humans (18) and rats (33), polyunsaturated acids derived from the 2-position of lecithin by LCAT predominate. Some HDL cholesteryl esters, however, may enter the circulation with nascent particles, in particular spherical particles of intestinal origin (67). A theoretical source of cholesteryl esters is a "reverse" cholesteryl ester-triglyceride transfer. This situation may operate to some degree immediately after the initiation of triglyceride-lowering therapy in hypertriglyceridemia.

Cholesteryl ester molecules leave the HDL particles rapidly in species whose plasma contains the core-lipid transfer reaction. For the sake of simplicity, the effects of the reaction on HDL-CE are depicted in Fig. 5 by three different pathways: CE ↔ CE exchange, CE ↔ TG bidirectional transfer process, and unidirectional transfer of CE molecules from HDL. The first pathway is a pure exchange process and, except for replacement of HDL-CE molecules by the same molecules in other lipoproteins, there is neither enrichment nor depletion of the HDL of cholesteryl esters. This pathway operates mainly between HDL and LDL. The two other pathways cause net loss of CE molecules from HDL. In our studies, we were able to remove from HDL_2 or HDL_3 as much as 50% of the CE molecules during incubation with VLDL and lipid transfer proteins (202, 203). An important but unresolved question is whether a triglyceride molecule replaces each molecule of CE transferred from the HDL. Such equimolecular bidirectional transfer of CE and TG molecules is expected to maintain the total number of core-lipid molecules in HDL constant. In Fig. 5, a pathway for loss of CE molecules from HDL without replacement by either CE or TG molecules is included. That pathway has been incorporated in part because of our own observations that demonstrated that possibility (198), and the recently described reaction of cholesteryl ester movement from different lipoproteins to tissue cells (210–212). Cholesteryl ester transfer to sphingomyelin liposomes has been demonstrated (208)

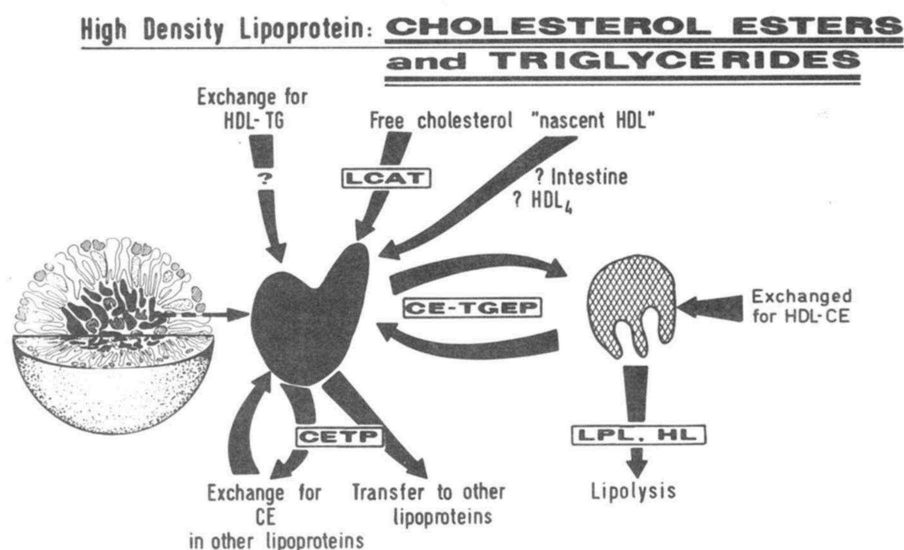

Fig. 5. Intravascular dynamics of HDL cholesteryl esters (CE) and triglycerides (TG). The main source of HDL-CE is the LCAT reaction. Theoretically CE may replace HDL-TG and most probably is a constituent of spherical nascent HDL particles of intestinal origin. In animal species whose plasma contains the lipid transfer proteins (CETP), HDL cholesteryl esters are exchanged with the same molecules in other lipoproteins and are transferred to triglyceride-rich lipoproteins. Some or all of the transferred CE molecules are replaced by triglycerides (CE-TGEP) and the transfer reaction is the major source of HDL-TG. HDL-TG is hydrolyzed by lipoprotein (LPL) and hepatic (HL) lipases. In the human, most or even all of the CE molecules in HDL are replaced by other molecules within 1 day.

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as well. Yet, in another study, it has been concluded that significant loss of CE molecules from HDL without replacement by other core molecules does not occur (213).

The lifetime of cholesteryl ester molecules in HDL is not clear. HDL contains between 1 and 1.5 mmol of cholesteryl ester in 1 liter of plasma. The total body HDL-CE pool then is about 4–8 mmol, an amount similar to that produced in 1 day by the LCAT reaction (70). In humans, most of the LCAT-derived cholesteryl esters are transferred to other lipoproteins. As mentioned above, some of these esters are possibly delivered directly to lower density lipoproteins, while other molecules are first assimilated in the HDL system and are transferred later. The contribution of each pathway to total CE transport is not clear. When free cholesterol is allowed to become esterified in rat plasma, at least 90% of the newly formed esters are recovered in HDL and less than 10% in other lipoproteins (33, 198). If this observation can be extrapolated to the human, then all of the LCAT-derived CE molecules should "turn over" through the HDL system in a day. The "residence time" of a cholesteryl ester molecule in HDL, therefore, is also 1 day or less.

The amount of triglyceride molecules in HDL is dependent on the presence and the rate of the lipid transfer reaction. Small amounts of triglycerides, however, may be present in nascent spherical HDL of intestinal origin (67), but not in discoidal particles. Intestinal origin probably accounts for the presence of triglycerides in the plasma of patients with abetalipoproteinemia (35). With that exception, most or even all HDL-triglycerides must be derived from triglyceride-rich lipoproteins by the lipid transfer reaction. Variation of the content and activity of lipid transfer proteins could be of importance in determining the number of triglyceride molecules in HDL. That probably does not occur. We have recently measured the activity of the core-lipid transfer reaction in normal human subjects and in patients with type IIA, IIB, and IV hyperlipoproteinemia (S. Eisenberg, unpublished results). Cholesteryl ester exchange between HDL and LDL was used to estimate total plasma activity. The rates of CE exchange ranged between 50 and 150 μmol/l/hr and were similar in all groups of subjects. Transfer activities were also recorded in one female subject with familial hyperalphalipoproteinemia, in three patients with abetalipoproteinemia, and in several samples of cord blood, although in the latter samples the activity was the lowest. It is therefore concluded that the most important single factor that determines the amount of triglycerides in HDL is the ratio between the mass of triglyceride-rich lipoproteins (chylomicrons and VLDL) and of HDL in the plasma. This conclusion is supported by the findings that TG to CE ratios in HDL are highly correlated with plasma triglyceride levels (214–216).

The fate of HDL-triglycerides is not clear. In vitro studies have demonstrated that lipoprotein lipases and hepatic lipases can hydrolyze HDL-triglycerides (203). To what extent HDL-triglycerides are susceptible to the activity of these enzymes in vivo is not known.

D. HDL apoproteins

HDL contains a host of apolipoproteins (A-I, A-II, A-IV, C-I, C-II, C-III, D, E, and F) and carries several other proteins, including the LCAT protein, the lipid transfer protein, the serum AA amyloid protein (217), and the beta-glycoprotein-I (218). The biological behavior of apoA-I and apoC has been studied extensively. In the following section the behavior of apoA-I, the major HDL apoprotein, will be discussed in detail, followed by a brief description of the behavior of other apoproteins.

1. Apoprotein A-I. In normal fasting human plasma, most of the apoA-I is present in HDL. For example, when ^{125}I-labeled apoA-I is added to human plasma or to a mixture of lipoproteins, about 95% of the radioactive protein is re-isolated with HDL (219). Radioimmunoassays of apolipoprotein A-I generally report that 80–90% of the immunoassayable apoA-I is present in HDL and the rest is found mostly with the plasma protein fraction of d > 1.21 g/ml (220, 221). Very small amounts of apoA-I are found with either LDL or VLDL. It has been suggested that apoA-I has a preferential avidity towards small particles with large radii of curvature (222–224). Yet, apoA-I is a major apoprotein of nascent chylomicrons (42–47). These observations are contradictory, unless chylomicrons possess specific binding sites for the protein. Even then, it is difficult to understand why chylomicrons release most of their apoA-I once they enter the circulation. One explanation for these contradictory observations is that the apoA-I present in chylomicrons is different from that present in plasma HDL. Thus, it is possible that nascent chylomicrons contain, in fact, the proprotein, and that conversion of pro-apoA-I to apoA-I occurs after the protein is released from the chylomicrons.

The intravascular dynamics of apoA-I (and other HDL apoproteins) are shown schematically in **Fig. 6.** Three potential sources for apoA-I are considered: nascent HDL particles, free apoA-I plasma pool, and apoA-I released from the surface coat of triglyceride-rich lipoproteins of intestinal origin. The first source is obvious and reflects the amounts of apoA-I that are associated with secreted spherical or discoidal HDL. The second is more complex. Small amounts of apoA-I (and other apoproteins) are present apparently as free

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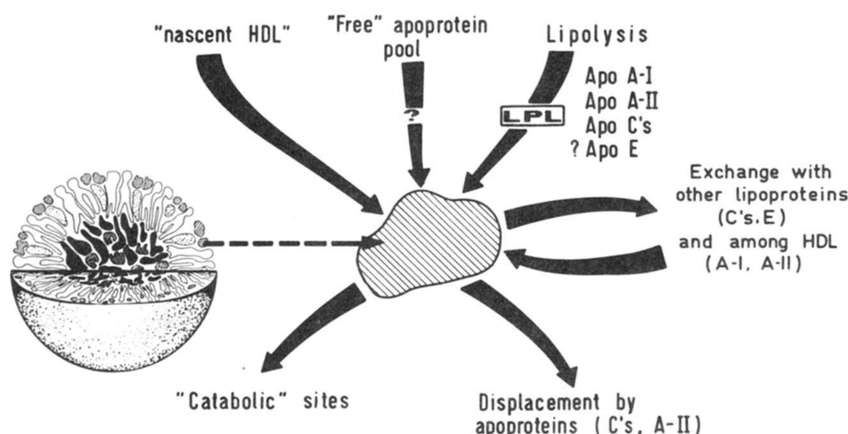

Fig. 6. Intravascular dynamics of HDL apoproteins. Apoproteins are part of "nascent" complexes and are generated during the lipolysis process. A small pool of rapidly turning over free apoproteins probably also contributes (see text). Once in an HDL particle, apoproteins rapidly exchange with other lipoproteins, including HDL. Some apoproteins may become displaced from HDL by other proteins (e.g., displacement of apoA-I by apoC or apoA-II). The apoproteins in HDL are responsible for the binding of HDL to specific cells and some of these interactions result in catabolic events (see Section VIII).

proteins (or are associated with very small amounts of phospholipids) in the plasma. The main evidence for the existence of a free apoprotein pool is the reported secretion of apoproteins in urine (225) and their presence in body fluids such as the cerebrospinal fluid (226). This free apoprotein pool conceivably represents apoproteins dissociated from lipoproteins and apoproteins secreted from cells that escape immediate association with lipoproteins. The third source is the surface coat of triglyceride-rich lipoproteins of intestinal origin. The mechanism(s) responsible for the release of apoA-I from chylomicrons (small or large) have not been completely clarified. Incubation of chylomicrons in plasma apparently does not result in significant release of apoA-I although both apoC and apoE are transferred to the chylomicrons (46, 227). In a preliminary study, we have not found appreciable exchange of apoA-I between small chylomicrons (biosynthetically labeled with [^{3}H]leucine) and rat HDL (T. Chajek-Shaul and S. Eisenberg, unpublished results). ApoA-I, however, is rapidly freed from the chylomicrons when the lipoprotein interacts with lipoprotein lipases (52, 151), and apoA-I content in HDL increases during clearance of alimentary lipemia (227, 228). The physicochemical basis for this process is not clear. For example, it is not known whether triglyceride hydrolysis is sufficient or whether some phospholipid hydrolysis is involved with this process.

Similar to other constituents, once apoA-I is present in HDL, the molecule does not remain associated with individual particles throughout their circulating lifetime. Rather, apoA-I molecules seem to be released and transferred between HDL particles. This again is accomplished by several different mechanisms. One mechanism is pure exchange process, i.e., replacement of an apoA-I molecule in an HDL particle by another molecule from another particle. This path has been studied mainly with HDL_2 and HDL_3 using ultracentrifugation (229), and gradient gel electrophoresis (230) to separate HDL species. Both apoA-I and apoA-II do exchange between HDL_2 and HDL_3 (229). Initial rates of transfer of radioactive proteins as high as 70%/hr have been reported. However, when the amounts of protein in two HDL fractions are similar, only 7–14% of the radioactivity is exchanged during a 5-hr incubation (230). In none of these experiments has a full equilibrium (equal specific activities of apoA-I in donor and acceptor particles) been achieved. Such equilibrium has been demonstrated for apoC (155). We have used another approach to study apoA-I and apoA-II transfer between HDL particles. In the study we used the lack of antigenic cross-reactivity between human and rat HDL as a tool to determine bidirectional transfer of human apoproteins to rat HDL and vice versa (231). Preferential transfer of human apoA-I to rat HDL was found, although some transfer of apoA-II was also detected. Of interest, only one-fourth to one-half of the human apoA-I was transferable under the conditions of the experiment (incubations of human and rat HDL's for 30 min followed by the addition of antiserum).

A second phenomenon is the exchange of free apoA-

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I in solution with the same molecule in HDL particles. These investigations were carried out to achieve labeling of the HDL with radio-iodinated apoA-I or apoA-II (232, 233). For both proteins, exchange between free molecules in solution and those associated with lipoprotein particles has been documented. With apoA-I, however, it was impossible to exchange all of the molecules present in HDL, and it has been concluded that part of the apoA-I in HDL is present as a discernible, unexchangeable pool. That conclusion agrees with our earlier observations (231).

The third mechanism of exchange is replacement of apoA-I molecules in HDL by other apoproteins. This phenomenon has been shown for apoC and apoA-II. In canine (234, 235) and human (236) HDL, addition of free apoA-II causes considerable or even complete replacement of apoA-I by A-II. The displaced apoA-I appeared as lipid-free protein or, in the presence of excess of apoA-II, as an apoA-I:apoA-II complex (235). Similarly, addition of apoC to human HDL results in accelerated displacement of apoA-I from the lipoprotein (237).

The physicochemical basis of apoA-I exchange has not been clarified. Grow and Fried (229) suggested formation of collision complexes. Pownall et al. (237) however, showed concentration-dependent dissociation of apoA-I from HDL when the lipoprotein is diluted in 0.15 M NaCl solution. This observation suggests spontaneous dissociation of apoA-I from HDL and agrees well with the concepts presented by Osborne and Brewer (238). Such a dissociation process accounts for the presence in aqueous solutions of a small pool of rapidly turning-over unassociated apoA-I. Such an apoA-I pool may adequately explain the different exchange phenomena described above, including displacement of apoA-I by other proteins. In this latter instance, the different affinities of apoproteins towards the lipoprotein surface would be critical in determining the final apoprotein profile of the lipoprotein. Finally, if such a pool exists in vivo, it may play an exceedingly important role in metabolic events. HDL conversions, discussed in Section VI, are examples where a changing apoprotein profile of HDL may occur.

2. Apoprotein C. Exchange and transfer of apoC among lipoproteins has been extensively reviewed in the past (9, 239). The proteins exchange rapidly between VLDL (and chylomicrons) and HDL (6, 7, 92, 95, 155, 219). This process is temperature-dependent (155), proportional to the mass ratio of the two lipoproteins (219), and is independent of phospholipid exchange (155). ApoC-II and apoC-III-1 behave similarly under a variety of experimental conditions (94). Whether the apoproteins exchange through a dissociation-association mechanism or lipoprotein collision has not been fully clarified. Although about 50% of plasma apoC mass is present in HDL (in normolipemic humans), the proteins apparently prefer the triglyceride-rich lipoproteins, as the available surface of VLDL and chylomicrons is only a small fraction of that of HDL. Yet C apoproteins displace apoA-I from HDL. A changing mass ratio between triglyceride-rich lipoprotein and HDL apparently explains the redistribution of C apoproteins during induction of alimentary lipemia (150) and after initiation of triglyceride hydrolysis by the injection of heparin to humans (7) or rats (90). It is significant to note that C apoproteins return to VLDL when newly secreted particles enter the plasma compartment (7). In that and other respects, therefore, the dynamics of apoC are very different from those of apoA-I.

3. Other apoproteins. The behavior of apoA-II is apparently very similar to that of apoA-I. Free apoA-II reassociates predominantly with HDL (219), can be incorporated into HDL (233–236), and exchanges between HDL_2 and HDL_3 (229, 230). Radioimmunoassays demonstrated apoA-II predominantly in HDL (240). Unlike apoA-I, however, apoA-II reassociates with both small and large lipid complexes (224) and has a higher affinity to lipoprotein surfaces (including HDL) than apoA-I. ApoA-IV is a prominent protein constituent of rat plasma HDL (241). In humans, the protein enters the plasma with nascent chylomicrons and is rapidly displaced to the lipoprotein-poor plasma fraction of d > 1.21 g/ml (242–244). Other apoproteins are probably associated with specialized HDL populations and do not reflect the behavior of the HDL system as a whole.

E. Dynamics of the HDL system

The data and considerations discussed in this section demonstrate the dynamic nature of the HDL system. In humans, none of the HDL constituents is associated with the particle for a long period of time. Each constituent, moreover, "turns over" in HDL with a different lifetime, ranging between a few hours to 1 day. In that respect, the HDL particle seems to play the role of a "temporary station" for lipid and proteins. Lipid constituents are transferred and exchanged into the particle, are used and modified by enzymes and transfer proteins and their metabolic products, or the remaining intact molecules are transported out of the HDL. The proteins behave similarly but remain unmodified. The role of protein movements is apparently to provide the HDL system with the necessary flexibility to accommodate different amounts of lipids and/or to supply proteins for intravascular synthesis of new HDL. The integrity of the HDL system appears to depend on and reflect the sum of all these movements and modifications of its constituents. In that sense, the HDL exists as a particle that provides a center of intense biological activity. The

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impact of this dynamic situation and the consequences of over- or under-activity of one or more of the biological reactions discussed above on HDL formation have been described in the previous section. The next section presents the effects of these processes on the levels, nature, and distribution of HDL subpopulations.

VI. REGULATION OF HDL SUBPOPULATION DISTRIBUTION

The data presented in Fig. 1 (composition of HDL populations) suggest that HDL particles can become larger or smaller when, and if, lipid and protein molecules are added or deleted from different particles. In the present section, the evidence that such conversions take place in physiologic situations, and their mechanisms, are discussed.

A. Conversion of HDL_3 to HDL_2

For this conversion to take place, it is necessary to increase the number of CE molecules in HDL_3 by 2- to 3-fold (from 40–50 to 100–120 molecules) and to provide HDL_3 with one molecule of apoA-I and sufficient amounts of surface lipids (phospholipids and free cholesterol). Available evidence suggests that the activity of the extrahepatic lipoprotein lipase system is strongly related to the levels of plasma HDL_2 in humans. HDL concentrations in plasma (as estimated by HDL-cholesterol) are in general proportional to the activity of lipoprotein lipase (103–106) and hence to rates of triglyceride transport (245). Because most or even nearly all the variation of plasma HDL concentration is explained by the variation of HDL_2 (246, 247), the relationship between HDL concentration and lipoprotein lipase activity reflects that of the HDL_2. A mechanism that explains these observations was proposed by us in 1978 and is graphically presented in **Fig. 7.** In a series of experiments, we have investigated the effects of VLDL lipolysis in vitro on the structure and composition of HDL_3 (248). The study has demonstrated that HDL_3 readily accepts phospholipids, free cholesterol, and apoproteins (predominantly apoC) released from the surface coat of the lipolyzed VLDL. By this process, the content of phospholipids and free cholesterol in HDL_3 increases by nearly 100%, and that of apoproteins by about 50%. The capacity of HDL_3 to accept phospholipids is saturated at that level and, when more phospholipids are released from VLDL, the molecules become attached to albumin (S. Eisenberg and J. Patsch, unpublished results). The capacity of HDL_3 to accept apoproteins is also limited; when a great excess of apoC is released from the VLDL, apoA-I molecules are displaced from the lipoprotein.

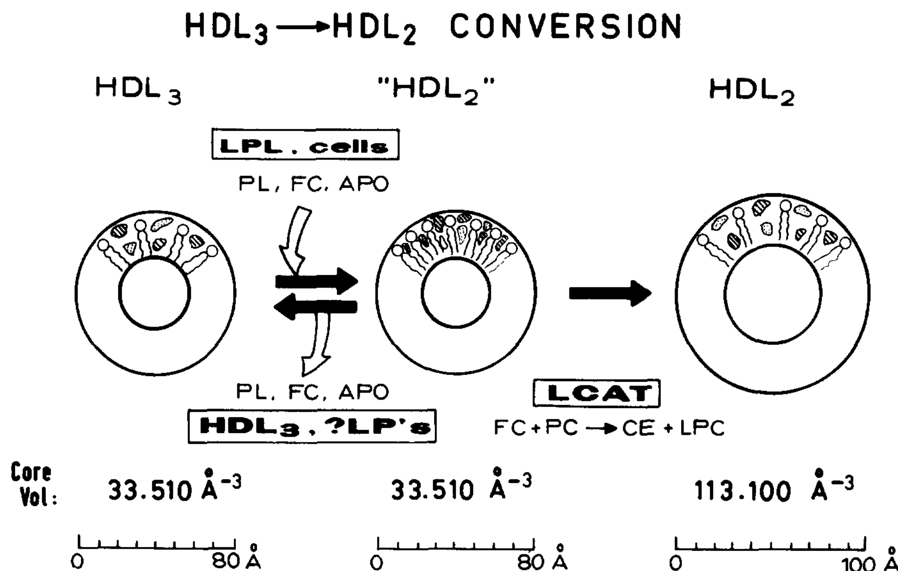

Fig. 7. A schematic representation of $HDL_3 \rightarrow HDL_2$ interconversion. The scheme suggests that the conversion of HDL_3 to HDL_2 is a two-step process. Lipolysis of triglyceride-rich lipoproteins, the surface coat of intact lipoproteins, and cell membranes supply HDL_3 with phospholipids, free cholesterol, and apoproteins. These molecules accumulate in existing HDL_3 particles and, if esterification does not occur, they may distribute to other lipoproteins or cell membranes. This step, therefore, is reversible. True HDL_2 formation depends on formation of cholesteryl esters via the LCAT reaction. "HDL_2" denotes the phospholipid- and free cholesterol-enriched HDL_3 and is possibly identical to the HDL_{2a} population described by Andersen et al. (246). An important feature of the $HDL_3 \rightarrow HDL_2$ conversion process is formation of thermodynamically favored HDL_2 particles (100 Å diameter) containing four molecules of apoA-I as discussed in the text.

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The conclusion that HDL_3 accepts lipids and proteins released from the surface of lipolyzed triglyceride-rich lipoproteins has been confirmed by other investigators (249) and agrees well with results recorded after initiation of lipolysis by the injection of heparin (7, 250) and during the clearance of chylomicronemia (150–152, 228). Yet, in our experiments when the LCAT system was either missing or inactive, we did not find a change in cholesteryl ester content or size of the HDL_3. Hence, we suggest that incorporation of molecules released from the surface of lipolyzed triglyceride-rich lipoprotein into HDL_3 is insufficient for complete conversion to HDL_2. It is therefore important to note that, although we have observed a shift of density of the HDL_3 toward the HDL_2 position, we considered that true HDL_2 cannot be formed unless the particles are enriched with cholesteryl ester molecules. The phospholipid- and free cholesterol-enriched HDL_3 thus should be considered as an "intermediate" in the path of $HDL_3 \rightarrow HDL_2$ conversion. Such HDL intermediates are possibly analogous to the HDL_{2a} population described by Anderson et al. (251) and are not very different from HDL_3 that becomes enriched with phospholipids after in vitro incubation with phospholipid vesicles (252–254). True conversion of HDL_3 to HDL_2 occurs only after the particle acquires cholesteryl esters, by the LCAT reaction. If that last reaction does not take place, the first part of the conversion path is fully reversible, as the surface lipids (and proteins?) added to the HDL_3 equilibrate with other HDL particles (S. Eisenberg and J. Patsch, unpublished results). Because of all these considerations, $HDL_3 \rightarrow HDL_2$ conversion is described in Fig. 7 as a two-step process, where the first step is potentially reversible.

The mechanism of $HDL_3 \rightarrow HDL_2$ conversion illustrated in Fig. 7 does not specify the source of apoprotein and lipids. In fact, lipolysis may be one of several sources. Cell membranes must be considered as another potential source of lipids that become associated with HDL_3 and contribute to the conversion process. The capacity of HDL_3 to accept cholesterol from cell membranes has been well established and is considered a major part of the "reverse cholesterol transport" process (see Section VIII). Whether phospholipids may leave cell membranes and become associated with HDL is not known. Finally, if apoprotein A-I and A-II are secreted from cells with no (or minimal amounts of) lipids, they may also contribute to the HDL conversion path rather than to the pool of HDL-precursors. Transfer of lipids (and proteins?) from cells to HDL probably accounts for the modifications that occur in HDL filtered through extravascular spaces (255).

The evidence that HDL_2 is formed in the plasma compartment by pathways not dissimilar to those described above is still indirect. The data that support the $HDL_3 \rightarrow HDL_2$ conversion have been mentioned above. An experiment that conclusively shows in vivo conversion of HDL_3 to HDL_2, however, has not been reported yet. Very recently, we decided to study this problem by following up human HDL_3 injected into rats (D. Gavish, Y. Oschry, S. Eisenberg, unpublished results). Preliminary results demonstrate conversion of the injected HDL_3 to HDL_2 and, later, to HDL_1. If these results can be corroborated in humans, that will be sufficient to verify the essential features of the $HDL_3 \rightarrow HDL_2$ conversion process. It is also possible, although less likely, that some HDL_2 is formed directly as a large-sized HDL population. As discussed below, mechanisms of conversion of HDL_2 to HDL_3 are also present in human plasma.

B. Conversion of HDL_2 to HDL_1 (apoE-HDL)

HDL_1 is an apoE-rich HDL population of lesser density and larger diameter than HDL_2. HDL_1 is a normal plasma lipoprotein constituent in the rat (31–33) and has been identified in human plasma (28–30). In rats, the contribution of apoE to total proteins in HDL_1 is estimated to be 60%, and the mean diameter of the lipoprotein is 130–140 Å (33). An analogous lipoprotein, HDL_c, accumulates in the plasma of most, or even all, animal species when the animal is given cholesterol-rich diets (34). Several recently published experiments indicate that HDL_1 is formed in plasma, presumably from HDL_2 (or heavier HDL populations). The origin of apoE in HDL is most probably lipolysis of triglyceride-rich lipoproteins (256–259). That alone suggests the intraplasma origin of HDL_1. More direct evidence for the plasma origin of HDL_1 is the observation that in vitro incubation of human plasma results in pronounced increase of HDL_1 levels, presumably due to accumulation of LCAT-derived cholesteryl esters in the lipoprotein (30, 260). Similar shifts of HDL_3 to HDL_1 were reported during incubation of canine plasma with Celite-coated cholesterol particles, and during clearance of cholesterol from lipid-loaded macrophages (261). More recently, we have studied the intravascular fate of the cholesteryl ester moiety of rat plasma lipoproteins (262). Because the rat is an animal whose plasma lacks the core lipid transfer activity (33, 193), shifts of cholesteryl esters between lipoproteins strongly suggest conversion of the lipoprotein itself. In the study, we consistently observed such shifts of HDL_2-cholesteryl esters to HDL_1. Very recently we found that human HDL_3 devoid of apoE also ends in the density range of HDL_1 after injection into rats (S. Eisenberg, unpublished results). These observations indicate that apoE-rich HDL_1 particles are formed in plasma from apoA-I-rich HDL_2 or even HDL_3 particles. In further support of this

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conclusion, are our earlier observations that the cholesteryl ester fatty acid profile of rat plasma HDL_1 indicates an LCAT origin of these esters (33). Thus, it seems that an $HDL_2 \rightarrow HDL_1$ conversion path exists in plasma and is possibly regulated by the same reactions that are responsible for the $HDL_3 \rightarrow HDL_2$ conversion.

Two important conclusions can be derived from these studies. The first concerns the ability of the HDL system to carry cholesterol. Conversions of small HDL particles to larger particles allow transport of several-fold more cholesterol in HDL, when needed. HDL_2 to HDL_1 conversion may provide a much larger capacity to transport cholesterol than is achieved by $HDL_3 \rightarrow HDL_2$ conversions. The need to transport even larger quantities of cholesterol in plasma is possibly responsible for accumulation of HDL_c in cholesterol-fed animals (34). The second conclusion concerns the different apoprotein profiles of HDL_2 and HDL_1. If the assumption that apoA-I-containing HDL is converted to apoE-containing HDL_1 is correct, then during this conversion apoA-I must be displaced from the HDL_2 and replaced by apoE. Graphic illustration of a hypothesis that accounts for such conversion is shown in **Fig. 8.** It is envisioned that in the rat most of the LCAT-derived cholesteryl esters remain in HDL. Hence, apoA-containing HDL in the rats circulates as HDL_2. With further accumulation of cholesteryl esters, some of the HDL_2 particles become even larger, and particles of the size, density, and composition of HDL_1 are formed. During this metabolic conversion, however, apoA-I molecules dissociate from the lipoprotein (probably due to the poor association of apoA-I with larger particles), and are replaced by apoE molecules. Because apoproteins serve as markers for interactions of lipoproteins with cells and enzymes, the metabolic significance of the changing apoprotein profile of HDL populations, if substantiated, cannot be overemphasized.

C. Conversion of HDL_2 to HDL_3

Several publications have suggested that HDL_2 particles may become depleted of lipids and proteins and form the smaller HDL population, HDL_3. Two pathways were proposed to cause this conversion. In the first, the role of the heparin-releasable hepatic lipase was emphasized, and in the second, that of the core lipid transfer reaction. The hepatic lipase has been shown repeatedly to hydrolyze HDL phospholipids and triglycerides in in vitro incubation systems (110, 163–166). Although lipids in other lipoproteins also serve as substrates for this enzyme (83–86, 163), it has been proposed that HDL lipids are preferable. It should, however, be pointed out that in in vitro systems HDL phospholipids can also be

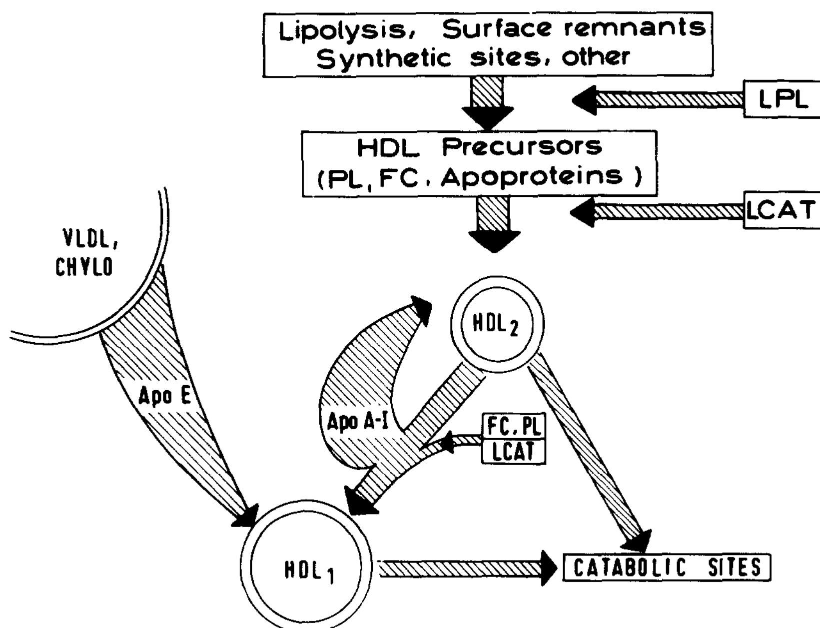

Fig. 8. A schematic representation of HDL_2 to HDL_1 conversion. The scheme is based on observations in the rat (33) and includes the events that are responsible for HDL_2 formation (lipolysis and cholesterol esterification). These same events cause further enrichment of the HDL_2 (diameter of ~100 Å) with cholesteryl esters and formation of large-sized HDL_1 (diameter ~140 Å) particles. Along this path, however, apoE molecules that originate from lipolyzed VLDL and chylomicrons displace apoA-I molecules from the growing HDL particles. By this process, apoE becomes the predominant apoprotein in HDL_1 while the displaced apoA-I is re-utilized for further formation of HDL_2.

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hydrolyzed by lipoprotein lipase (108, 109). When we determined the activity of lipoprotein lipase and hepatic lipase against HDL phospholipids, we found that with the hepatic lipase we were able to hydrolyze 2- to 3-fold more HDL-phospholipids than with lipoprotein lipase (S. Eisenberg, unpublished results). In a recently published study, human serum was incubated with hepatic lipase and the change of lipoprotein profiles was followed (263). The enzyme hydrolyzed phospholipids and triglycerides in VLDL, LDL, and HDL with preference towards both VLDL and HDL. When HDL was fractionated, a marked decrease of HDL_2 and increase of HDL_3 mass was found. It has thus been proposed that as a result of the activity of the hepatic lipase, HDL_2 is converted to HDL_3 (263). The conclusion that the hepatic lipase affects HDL metabolism (as well as other lipoproteins) is supported by several additional unrelated observations. Firstly, administration of specific hepatic lipase antiserum to rats (264–266) or primates (267) results in marked increase of HDL phospholipid and possibly triglyceride levels. Secondly, administration of heparin to human patients with lipoprotein lipase deficiency releases hepatic lipase and reduces HDL_2 phospholipid and protein content, and increases HDL_3 phospholipids (268). Thirdly, strong negative correlations are found between the levels of HDL and those of hepatic lipase (269, 270). Strong associations have also been reported between the increase of the hepatic lipase activity and the decrease of HDL_2 levels in women taking progestins with androgenic activity, while estrogens that decrease the hepatic lipase activity caused increased HDL_2 levels (271–275). Estrogens, however, also increase apoA-I synthesis (see Section VII). Finally, remarkably high HDL_2 levels were reported in two human patients with hepatic lipase deficiency (276).

The data described above strongly support the concept that the hepatic lipase plays an important role in HDL metabolism and is involved with processes that cause "reverse conversions" of HDL, i.e., formation of smaller particles from larger particles. Yet the main feature that distinguishes HDL_2 from HDL_3 is their content of cholesteryl esters. A true conversion of HDL_2 to HDL_3, therefore, must be associated with loss of cholesteryl esters from HDL_2. In the study of Groot, Scheek, and Jansen (263) for example, the shift in density of HDL_2 towards HDL_3, observed after incubation of human plasma with hepatic lipase, may simply reflect hydrolysis of lipids and not a true conversion of HDL_2 to HDL_3. Our approach was to seek metabolic pathways that are responsible for removal of core lipids from large-sized HDL populations. Our first study was carried out in plasma obtained from patients with abetalipoproteinemia, a disease where abnormally large-sized HDL particles are found (35). Addition of VLDL to such plasma induced transfer of cholesteryl esters from HDL to VLDL and of triglycerides from VLDL to HDL. Yet the HDL retained its size and density. However, when the incubation system was supplemented with lipoprotein lipase and HDL lipolysis was allowed to take place (after removal of the VLDL), a decrease in the diameter and the density of the lipoprotein was observed. This reaction was studied by us more recently in greater detail while using human plasma HDL_2 and a variety of in vitro incubation procedures (203). The study demonstrated that particles similar to HDL_3 (by composition, size, and density) can be formed from HDL_2 when the activity of the core lipid transfer proteins is combined with lipolysis of the transferred triglycerides. That conversion was independent of the source of triglyceride-rich particles (VLDL or Intralipid), the source of transfer activity (lipoprotein-deficient plasma or partially purified lipid transfer proteins), and the source of lipolytic enzymes (lipoprotein lipase or hepatic lipase). In vivo, however, it is reasonable to assume that the hepatic lipase plays a dominant role in this process.

Fig. 9 presents a hypothesis that combines the activities necessary for $HDL_2 \rightarrow HDL_3$ conversion. This hypothesis is applicable in general to situations that favor formation of small HDL particles from larger particles. A two-step process is proposed. The first step involves the activity of the plasma lipid transfer protein and causes replacement of HDL-cholesteryl ester by triglyceride molecules. Triglyceride-rich lipoproteins serve as both cholesteryl ester acceptors and triglyceride donors. It is suggested that the cholesteryl ester-poor, triglyceride-rich HDL serves as intermediate in the conversion process. The second step is triglyceride hydrolysis and deletion of core lipid molecules from the HDL. It is in this stage of the conversion process that the hepatic lipase plays a major role, although lipoprotein lipases may also contribute. An essential feature of the second step is removal of surplus surface constituents, a process that may involve phospholipid hydrolysis by the hepatic lipase followed by exclusion of free cholesterol and apoprotein molecules. In the circulation, the two steps probably take place concomitantly, and as triglyceride molecules replace cholesteryl esters, they are hydrolyzed by lipases, and the particle loses core lipid molecules. A "quantum jump" may take place with the exclusion of an apoA-I molecule at a favorable thermodynamic status of the particle, as described below.

D. Regulation of HDL subpopulation distribution: role of enzymes and transfer proteins

In the foregoing discussion, the role of enzymes and of lipid transfer proteins in HDL conversion processes has been described. Other metabolic pathways that affect HDL conversion are generation and availability

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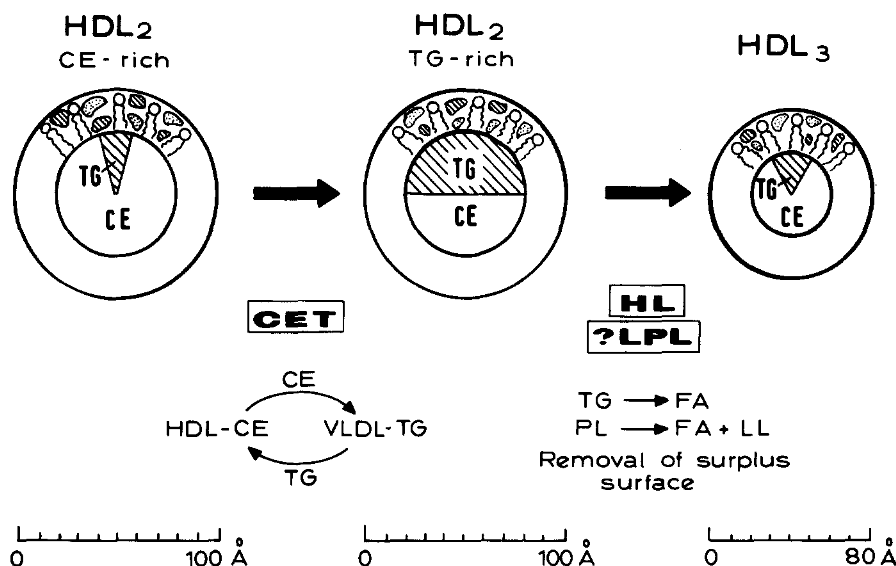

Fig. 9. A schematic representation of HDL_2 to HDL_3 conversion. This again is a two-step process. The first step depends on the lipid transfer reaction (CET) and replacement of part of the HDL_2 cholesteryl ester molecules by triglycerides. VLDL (and chylomicrons) participate in this reaction as acceptors for cholesteryl esters and donors of triglycerides. During this remodelling step, the size and density of the HDL_2 does not decrease; a transient increase may occur. Hydrolysis of the transferred triglycerides (predominantly by the hepatic lipase (HL) but also by lipoprotein lipases (LPL)), however, results in decreased size and density of the lipoprotein. Continuing replacement of cholesteryl ester by triglycerides and triglyceride hydrolysis coupled with removal of surplus surface (? phospholipolysis followed by displacement of apoproteins) results in complete conversion of HDL_2 to HDL_3.

of phospholipids and free cholesterol, cholesterol esterification, availability of apolipoproteins, and sources and relative masses of triglyceride-rich lipoproteins that participate in the lipid transfer reaction. The nature of interactions and affinities of the enzymes and transfer proteins with different lipoproteins is essential for the regulatory processes. Only when the coordinated effects of all reactions are considered, can HDL subpopulation distributions be properly analyzed. The activity of the LPL and LCAT systems, coupled with a proper supply of phospholipids, free cholesterol, and apoproteins, results in the predominance of large-sized HDL populations. The activity of the hepatic lipase and the core lipid transfer proteins, in the presence of a relatively large mass of triglyceride-rich lipoproteins, acts in the opposite direction. The HDL system, therefore, reflects the equilibria of these opposing pathways, as suggested originally by us while studying the plasma lipoproteins in abetalipoproteinemia (35). In this condition, chylomicrons, VLDL, and LDL are absent from plasma. HDL levels are low and there is an abnormally large-sized HDL population (diameter of 130–140 Å). Apoprotein turnover studies demonstrated normal catabolism of apoA-I, with reduced synthetic rates (277). Lipoprotein lipase and hepatic lipase activities are moderately decreased (278) and the lipid transfer proteins are present (S. Eisenberg, unpublished results). LCAT is also present. The main determinant of the HDL subpopulation distribution, therefore, seems to be absence of lipoprotein acceptors for transferred cholesteryl esters and donors of triglycerides.

Fig. 10 has been prepared to illustrate the pathways that regulate the HDL subpopulation distribution in normal subjects and patients with abetalipoproteinemia (35). The pathways included in Fig. 10 and in Figs. 7–9 also provide a physiologic background for the alteration of the HDL system in many other conditions. It is worthwhile to discuss briefly other examples that illustrate different determinants of HDL subpopulation distribution. In rats, HDL exists only as large-sized populations, HDL_2 and HDL_1, compatible with the absence of the core-lipid transfer reaction (33). Another example is the HDL system in patients with severe hypertriglyceridemia due to lack of lipoprotein lipase activity. HDL in such patients is exclusively in the HDL_3 range; it is denser than normal HDL_3 and is cholesteryl ester-poor and triglyceride-rich (279, 280). Limited availability of "surface remnants" and the presence in plasma of an extremely large mass of triglyceride-rich lipoproteins that accelerates core-lipid transfer together with normal,

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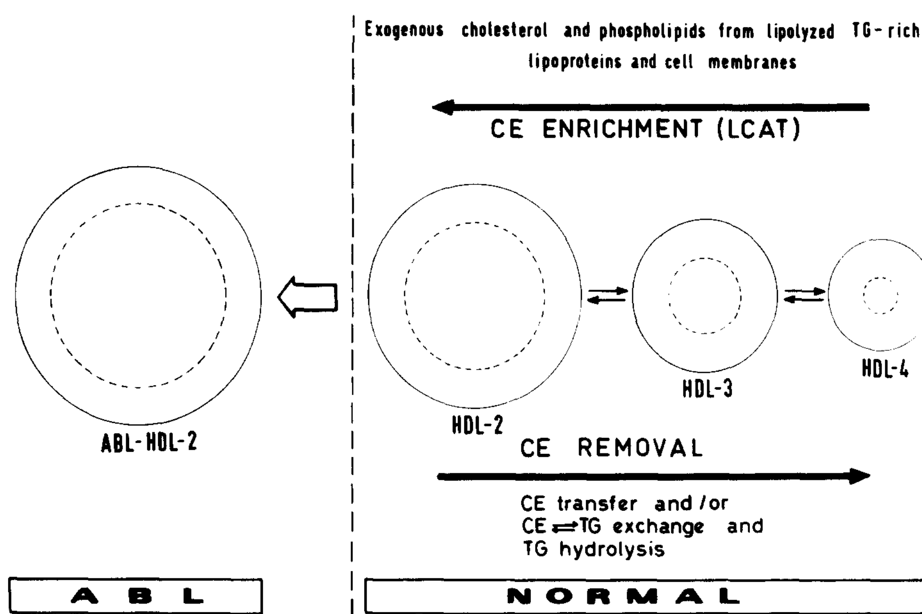

Fig. 10. An integrated scheme of the regulation of HDL subpopulation distribution. The scheme is based on observations in normolipidemic and abetalipoproteinemic subjects (35) and is reproduced (by permission) from the Journal of Lipid Research (1982. *J. Lipid Res.* **23:** 1280). The scheme predicts that HDL subpopulation distribution is determined by a balance of processes responsible for enrichment and removal of cholesteryl esters from HDL, as detailed in Figs. 7–9.

or close to normal, hepatic lipase activity probably accounts for the abnormalities of HDL in these patients. Finally, the well-known inverse relationship between plasma triglyceride and HDL levels and the predominance of HDL_3 in subjects with milder forms of hypertriglyceridemia are also determined by cholesterol and triglyceride redistribution and lipolysis of the transferred triglycerides (215, 216).

None of the metabolic pathways discussed in this section and summarized in Figs. 7–10 explain the presence of discrete, separable HDL subpopulations in plasma. It is thus not clear why HDL should not distribute continuously and symmetrically along the density range of 1.063–1.21 g/ml. In fact, microheterogeneity of HDL_2 and HDL_3 has been documented (26) and it appears that particles exist within each population of slightly higher and lower density, size, and composition. We have suggested that addition or deletion of one apoA-I molecule is responsible for transformations between HDL_2 and HDL_3 (15, 16). Our assumption is that two thermodynamically favored conditions occur when the HDL particle contains three (HDL_3) or four (HDL_2) molecules of apoA-I. According to this view, HDL_3 acquires few cholesteryl ester molecules by the LCAT reaction. That accounts for the microheterogeneity of the lipoprotein. Concomitantly, phospholipid and free cholesterol are added to the surface layer. At a certain stage, one apoA-I molecule is also added. That accelerates accumulation of cholesteryl esters and results in the "quantum jump" to HDL_2. HDL_2 microheterogeneity (towards larger (HDL_1) or smaller (HDL_3) particles) can of course exist until a further conversion or "reverse-conversion" occurs.

VII. INTRAVASCULAR METABOLISM OF HDL APOLIPOPROTEINS

The considerations presented above indicate that none of the HDL lipids or proteins can be employed as a "stable plasma marker" to study the intravascular metabolism of HDL particles in humans (or other species whose plasma contains core-lipid transfer activity). The fate in plasma of apoA-I and apoA-II therefore will be described with care in order not to equate the protein kinetic parameters with those of the lipoprotein.

Blum et al. (281) were the first to report kinetic parameters separately for apoA-I and apoA-II; in earlier studies, total HDL protein metabolism was investigated (282–284). In the study of Blum et al. (281), the whole HDL particle (d 1.09–1.21 g/ml) was radioiodinated, and the decay of labeled apoA-I and apoA-II was

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followed separately. The plasma decay of apoA-I paralleled that of apoA-II, and the mean circulating half-life of either protein was 5.8 days. Synthesis rates of HDL proteins were 8.0 mg/kg per day in males and 9.3 mg/kg per day in females. Mathematical modelling of plasma radioactivity decay was consistent with degradation from two compartments: a plasma and a nonplasma compartment. Rates of catabolism of HDL apoproteins from those two compartments were generally in opposite directions. Diets rich in carbohydrates caused increased catabolism of HDL proteins, whereas administration of nicotinic acid caused a decrease. Neither the diet nor the drug had appreciable effects on synthetic rates. Schaefer et al. (285–287) used a different technique to measure HDL apoprotein metabolism: injection of isolated labeled apoA-I or apoA-II. Plasma decay after injection of isolated radiolabeled apoproteins was similar, or even almost identical, to the plasma decay of the apoproteins after injection of labeled HDL particles (287). However, simultaneous injection of apoA-I and apoA-II (labeled with two isotopes) revealed that the plasma residence time of apoA-I (4.46 days) was significantly shorter than that for apoA-II (4.97 days). ApoA-I and apoA-II synthetic rates were significantly higher in females as compared to males (13.6 and 11.1 mg/kg per day for apoA-I and 2.5 and 2.1 mg/kg per day, respectively). The plasma or whole body residence time of either protein was similar in males and females. ApoA-I and apoA-II concentrations, total HDL and HDL_2 (but not HDL_3) mass, and HDL-cholesterol, -protein, and -phospholipid (but not HDL-triglyceride) were all positively and significantly correlated with apoA-I catabolism (measured as residence time). Negative correlations were found between plasma triglyceride or VLDL cholesterol levels and apoA-I or A-II catabolism. The synthesis and catabolism of apoA-I and apoA-II were highly correlated. Surprisingly, the correlation between apoA-II catabolism and most of the other measured parameters did not reach a level of significance, although the same trends as found for apoA-I were observed. Synthetic rates of either apoprotein were not correlated with any of the measured parameters. Of particular interest was the observation that apoA-I catabolism is greatly accelerated in patients with Tangier disease, with a mean plasma residence time of 0.52 days (285). Estrogens increased apoA-I synthesis by 25%, while no change was observed in catabolism in spite of decreased hepatic lipase activity (288). ApoA-II kinetics remained unchanged. A different methodology for the study of HDL apoprotein kinetics was developed by Shepherd et al. (232, 233). These investigators labeled HDL with radioiodinated apoproteins by exchange and re-injected the HDL into the circulation. With this method, the plasma decay of the exchanged apoA-I was 20–25% faster than apoA-I labeled in holo-HDL; the decay of apoA-II was similar (289). Using the in vitro exchange procedures, the following findings were reported. High polyunsaturated fat diet decreased HDL levels (especially HDL_2) and apoA-I synthetic rate, while no change was found in apoA-I catabolic rate (290). Nicotinic acid therapy increased apoA-I levels by decreasing its catabolic rate but decreased apoA-II synthesis and plasma levels (291). Cholestyramine also increased plasma apoA-I levels but this was due to increased synthesis (292). No change was found for apoA-II. Interestingly, no significant differences of apoA-I and apoA-II metabolism were found between males and females (293).

Fidge et al. (294) studied apoA-I and apoA-II metabolism in 4 normal and 16 dyslipoproteinemic subjects (types 2a, 2b, 4, and hyperalphalipoproteinemia). The subjects were injected with ^{125}I-labeled HDL and apoproteins were separated by SDS-PAGE (294). The circulating half-lives of apoA-I and apoA-II were 3.7 and 4.4 days, respectively (mean of all studies). The data were further analyzed by a two-pool model, with pool 1 being predominant. ApoA-I mass in pool 1 was negatively correlated with the irreversible removal rate and positively with production rates (flux through pool 1). ApoA-I flux through pool 1 was positively correlated with body weight and irreversible removal rate and negatively with LDL-apoB concentration. Similar results were obtained for apoA-II. Apparently, the rate of HDL catabolism was delayed in the presence of a high LDL-apoB plasma concentration. Of particular interest was the observation that apoA-I flux was strongly related to VLDL-apoB flux, determined in 10 subjects. The basis for these correlations is unclear. In another study (295), accelerated catabolism of apoA-I was found in vegetarians, without significant change of flux. Catabolism appears to account for the lower apoA-I levels in these subjects.

HDL apoprotein metabolism was also reported by Rao et al. (296) and Magill et al. (297). The first study included 10 normo- and 11 hypertriglyceridemic subjects (296). In normal subjects, apoA-I and apoA-II specific activities decayed from plasma in parallel (FCR's 0.247 and 0.225, respectively), but in hypertriglyceridemic subjects, apoA-II decayed much faster than apoA-I (FCR's of 0.252 for apoA-I and 0.340 for apoA-II). The apoA-II FCR was linearly correlated to plasma triglyceride levels. Comparing hypertriglyceridemic to normal subjects, apoA-I synthetic rates were lower and apoA-II catabolic rates were higher. There were no differences of apoA-I catabolism or of apoA-II synthetic rates. In the second study (4 normal and 13 hyperlipemic subjects, types I, IIa, IIb, IV, and V) VLDL-apoB kinetics and adipose tissue lipoprotein lipase activities

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were determined as well (297). The results recorded in the study differed in part from those reported by Fidge et al. (294). Whereas no relationships were found between VLDL-apoB synthetic rates and apoA-I synthesis, the two were highly correlated for apoA-II. ApoA-I, A-II, and HDL cholesterol levels were all positively and significantly correlated with VLDL apoB fractional catabolic rates. As expected, the fractional catabolic rate of either apoA-I or apoA-II was negatively correlated with that for VLDL-apoB. These findings indicated that the variance of HDL components concentration was associated with changing catabolic rates rather than synthesis rates.

The data described above allow some insight into the physiology and pathophysiology of HDL apoprotein metabolism, but leave several important questions unanswered. Firstly, a small but consistent difference was found in most studies between the rates of catabolism of apoA-I and apoA-II. This possibly reflects the presence in plasma of at least two HDL populations, such as the A-I-containing HDL and the A-I/A-II HDL (37, 38), or HDL_2 and HDL_3. Disparity between studies may thus reflect the predominance in plasma of one of the populations. Of course, other possible explanations to this phenomenon cannot be ruled out. Secondly, in many instances apoA levels seem to depend on rates of catabolism rather than synthesis. This is not surprising since apoA-I (and A-II?) synthesis, in particular by the intestine, should be independent of most if not all metabolic events that occur in the plasma. The exception to this rule, male:female differences that are apparently determined by synthetic rates, probably reflects the known effects of sex hormones on protein synthesis. Thirdly, correlations are consistently found between apoA metabolism and the metabolism of other lipoproteins and apoproteins, mainly VLDL, LDL, and apoB. These associations, although poorly understood, are also consistent with the view that all plasma lipoproteins are affected by similar metabolic pathways. Obviously, however, many more studies in humans are needed to elucidate events that affect the intravascular metabolism of HDL apoproteins.

VIII. HIGH DENSITY LIPOPROTEIN CATABOLISM

In spite of considerable investigational efforts, the tissue sites and mechanism of degradation of HDL are still obscure. Unlike LDL with its specific and unique receptor, HDL appears to have several modes of interaction with cells. Some of the interactions are reversible and not followed by catabolic events. Others are followed by influx or efflux of cholesterol to or from the cells, while protein degradation is observed after still other interactions. In addition, the nature of interaction of HDL with cells differs between tissues. Finally, HDL preparations often contain apoE-HDL and experimental observations become uninterpretable. With these reservations in mind, data on HDL degradation in intact animals will be discussed first, followed by a discussion of the interactions of the HDL molecule with various cell types in culture.

A. Studies in intact animals

Early studies were carried out with ^{125}I-labeled rat HDL (d 1.085–1.21 g/ml) (298–301) or with ^{125}I-labeled rat apoA-I (257). The circulating half-life of apoA-I in rats ranged between 10 and 11 hr. Accumulation of radioactivity in liver and intestine has been documented although some radioactivity was found in most tissues (298, 299). Similar results were reported later by other investigators (302–305). Using radioautographic techniques, Rachmilewitz et al. (300) demonstrated concentration of radioactivity over lysosomes, predominantly in hepatocytes. Nonparenchymal cells contained few radioautographic grains. More precise quantitation of the contribution of different tissues to HDL degradation was studied with nondegradable (or slowly degradable) molecules. Stein et al. (306) used HDL labeled with cholesteryl-linoleyl ether and showed that liver and carcass were the predominant sites of HDL degradation. Highest specific activity (counts/g of tissue), however, was found in the adrenals. In estrogen-treated rats even higher proportions of the ethers were found in liver, intestine, and adrenals. In the liver, the ethers were apparently contained in parenchymal cells. Glass et al. (307) also used cholesteryl ethers but in addition exchanged into the HDL apoA-I labeled with covalently linked ^{125}I-labeled tyramine cellobiose which accumulates in cells degrading the apoprotein. Plasma decay of apoA-I was slower than the cholesteryl ether by 20–30%, confirming the results reported by Stein et al. (306). The distribution of ethers among tissues demonstrated predominance in the liver, with 93–96% of the radioactivity associated with hepatocytes. Highest specific activity was found in adrenals, followed by ovaries and liver. The tissue distribution of apoA-I was distinctly different. While the liver remained predominant, it trapped far less apoA-I than cholesteryl ethers. Very high amounts of apoA-I were found in the kidney, an organ that degraded only trace amounts of ethers. Adrenals and ovaries accumulated only small amounts of apoA-I. Comparison of tissue uptake of cholesteryl ether to apoA-I demonstrated very high ratios in adrenals and ovaries (7.0 and 4.6, respectively), moderately high ratio in liver (2.3), and very low ratio in kidney (0.03). With other tissues the ratio ranged between 0.9–1.4, as

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expected for similar rates of catabolism of proteins and lipids. If the protein labeling technique does not modify the metabolic behavior of apoA-I, the study indicates that a significant fraction of the HDL is not degraded as a whole particle. As well, the study suggested that some tissues (i.e., adrenal and ovaries and perhaps liver) preferentially extract cholesteryl esters from HDL while the kidneys may avidly degrade filtered unassociated apoA-I, as previously suggested by these investigators (308).

Preferential uptake of cholesterol from HDL by adrenals and ovaries in rats has also been demonstrated by Andersen and Dietschy (309, 310). These investigators measured the effects of infused human and rat lipoproteins on cholesterol content and synthesis in rats treated with 4-aminopyrazolopyrimidine (4-APP). Such rats have very low plasma cholesterol levels, and rates of cholesterol synthesis in most tissues are maximal. In both adrenal and ovaries, HDL rather than LDL appeared to be the major source of cholesterol. More recently, specific binding and clearance of rat and human HDL by adrenal, ovaries, and liver has been demonstrated (311). The liver clears at least 24% of the HDL. The mechanism of transfer of cholesterol to tissues, however, was not studied and it is possible that cholesterol molecules rather than HDL particles are taken up.

B. Interactions of HDL with cells in culture

Because different cells interact differently with HDL, the metabolism of HDL by cells in culture will be discussed by cell types.

1. Hepatocytes. High affinity binding, uptake, and internalization of HDL by parenchymal and nonparenchymal liver cells in culture has been reported by several investigators (312–321). The two cell types bind and degrade HDL. Norum and associates (313–318) reported higher intake in parenchymal than nonparenchymal cells, while others reported the opposite (319–321). Parenchymal cells predominate in in vivo experiments (300, 306, 307). HDL without apoE was not used and it is possible that the cell culture data reflect in part apoE or B, E receptor activities (321). Because only part of the bound HDL was degraded, it has been suggested that internalization of the lipoproteins is a rate-limiting step of their intracellular degradation (321). The binding of rat HDL to hepatocytes is competitively inhibited by rat and human VLDL, LDL, and HDL (312, 318), is pronase-sensitive but does not require divalent cations, is not inhibited by heparin and apoE, and is not sensitive to modification of the HDL by cyclohexadione or neuraminidase treatment (318). An HDL binding site clearly distinct from the apoE or B, E receptors is therefore present. Lysosomal inhibitors (chloroquine, leupeptin, and ammonia) decrease HDL degradation by about 50%, indicating that lysosomes play an important role in this process (316). The cells degrade HDL proteins (312, 314, 315, 318) and cholesteryl esters (313, 322), but quantitative analysis of these processes has not been carried out. Similar results were also reported for rabbit hepatocytes and rabbit lipoproteins (323, 324).

Uptake and degradation of apoE-free HDL by cultured porcine hepatocytes has been reported more recently (325). Binding, internalization, and degradation of the apoE-free HDL were inhibited by unlabeled HDL but also by LDL, methyl-LDL, and methyl-HDL. More HDL than LDL was bound to the cells. Lysosomal inhibitors inhibited degradation. HDL binding and degradation, however, were unaltered by pronase treatment or preincubation of the cells with medium containing HDL or LDL, treatments that inhibit LDL catabolism. The authors concluded that porcine hepatocytes possess a specific site that recognizes both LDL and apoE-free HDL and is responsible for degradation of HDL but little, if any, LDL. Most of these conclusions were recently corroborated in a rat hepatoma cell line (326). Interestingly, in the former study (325), pre-incubation of the hepatocytes with medium containing HDL increased LDL degradation by about 60%. This phenomenon probably reflects cholesterol efflux from the cells.

The possibility that hepatocytes degrade HDL but also preferentially extract cholesteryl esters from the lipoprotein was recently investigated by Glass et al. (327) and Leitersdorf et al. (328). Hepatocyte and adrenal cells were used in the two studies and accumulation of cholesteryl-linoleyl ether was compared to apoprotein degradation. Glass et al. (327) reported that the uptake of cholesteryl ether was 8-fold that of apoA-I in hepatocytes and up to 40-fold in adrenal cells. Leitersdorf et al. (328) used HDL (d 1.085–1.21 g/ml), HDL_2 (prepared by zonal centrifugation), and apoE-free HDL. The ratio between uptake of the ether molecules and apoprotein degradation in hepatocytes ranged between 1.00 to close to 3.0 with the lower values observed with higher concentrations of HDL. In adrenal cells, the ratios were between 2.0–3.0. When ACTH was added, uptake of ethers and degradation of proteins were both stimulated, more so the former, and the ratio between the two increased to about 5.0. The two studies therefore demonstrated the complexity of HDL catabolism by cells. Some HDL apparently is taken up and degraded as a lipid-protein complex, while cholesteryl esters are preferentially extracted by the cells without catabolism of the protein moiety. This last process is more pronounced in adrenal cells and is stimulated by ACTH. Whether the process of cholesteryl ester transfer from HDL to cells is similar to that described for triglyceride-rich lipoproteins (210–212) is not known. It is, however,

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significant that Leitersdorf et al. (328) have not found a role for the hepatic lipase in this process.

2. *Adrenal and other endocrine cells.* This subject has been recently reviewed in detail (329). The in vivo and tissue culture experiments cited above demonstrate that HDL is an important source of cholesterol to the adrenals and ovaries in the rat and that that process is stimulated by ACTH (306, 307, 309–311, 327, 328). These results generally confirm the earlier observations reported by Gwynne and associates (see ref. 329). Similar data were reported for the mouse (330). In humans and primates the adrenal apparently does not use HDL (329, 331). One mechanism that accounts for adrenal uptake of HDL cholesterol is high affinity binding of the HDL to specific receptors followed by degradation of the lipoprotein (329, 332, 333). Another mechanism discussed above is preferential uptake of cholesteryl ester molecules (327, 328). In the rat, this latter mechanism apparently predominates.

The observations in the luteinized rat ovaries are similar to those described for the adrenals (329). Like the adrenal, ovarian cells use HDL cholesterol in the rat but not in other species (329). The cells possess specific binding sites for HDL (329, 334–338) that are regulated by gonadotropins (334–338). Again, the possibility that substantial amounts of HDL-cholesterol are delivered to the cells independently of apoprotein degradation has been suggested (329). HDL metabolism by testicular and placental cells has been studied infrequently. Specific binding of HDL by the two tissues has been reported but degradation is negligible (329, 339, 340). It is also uncertain how much HDL-cholesterol is supplied to these cells, if any.

3. *Intestine and kidneys.* Nestel, Fidge, and co-workers (341–343) investigated the interaction of HDL (and LDL) with enzyme-dispersed rat intestinal mucosal cells. Human HDL and apoE-free rat HDL bound to specific saturable cell receptors. Binding was nearly noncompetitive by either human or rat LDL, but was inhibited by excess of HDL. Binding was followed by internalization and degradation. Chloroquine reduced degradation and more HDL was associated with the cells, indicating that lysosomes were involved. Comparison of intestinal, adrenal (342), and liver cells (343) indicated that uptake of HDL is related to catabolic events in the intestine and liver whereas, in the adrenals, the receptosomes may escape fusion with lysosomes. As in previous studies, ACTH stimulated HDL binding to the adrenal 5-fold, but degradation was only slightly affected.

The role of the kidney in HDL apoprotein metabolism was reported in an abstract (344). The investigators demonstrated that the rat kidney possesses high-affinity specific binding sites for rat HDL and rat apoA-I, but not human HDL. Binding to kidney membranes was unaffected by EDTA, and both HDL lipid and proteins were found in the membrane, indicating binding of the whole HDL particle. Human LDL and HDL did not compete for this binding site, and rat HDL rich or poor with apoE was found to bind with similar affinity. In another abstract (345), accumulation of radioactivity in kidneys and liver was demonstrated in rats treated with the lysosomal protease inhibitor leupeptin.

4. *Fibroblasts.* Earlier studies with human fibroblasts demonstrated that HDL or plasma from patients with abetalipoproteinemia did not down-regulate the activity of HMG-CoA reductase (346, 347). A few years later, Miller, Weinstein, and Steinberg (348) reported that while binding of human HDL (d 1.09–1.21 g/ml) to fibroblasts is similar to that of LDL, internalization and degradation are about one-tenth. HDL degradation, in fact, was only slightly above that expected by fluid pinocytosis and invagination of plasma membranes (348). Binding was not affected by pre-incubation of the cells with lipoprotein-deficient serum, 7-ketocholesterol, or cycloheximide, and was resistant to mild proteolytic digestion (349). In contrast to LDL, almost no differences were found between fibroblasts obtained from normal subjects or from patients with familial homozygous hypercholesterolemia (350). These detailed studies indicated that HDL binding to fibroblasts is distinctly different from LDL and is not followed by internalization and degradation of the particle. Uptake and degradation of rat HDL (d 1.085–1.21 g/ml) by cells in culture (351) and differences between human and rat HDL (352) probably reflect the presence of apoE in rat lipoproteins.

The nature of HDL binding to fibroblasts and smooth muscle cells has recently been investigated by Biesbroeck et al. (353); binding of apoE-free total HDL, HDL_2, HDL_3, and VHDL (d 1.21–1.25 g/ml) was determined. With all four preparations, low-affinity, non-saturable binding ("nonspecific") and high-affinity, saturable binding components were identified. The high-affinity binding saturated at about 20 μg of protein/ml and was observed in LDL receptor-negative cells. Competition experiments demonstrated that all HDL preparations (including apoE-free HDL) competed for the same binding sites, while LDL was a weak competitor for HDL binding. Sterol-depleted HDL_3 was almost as effective as untreated HDL_3. In contrast to LDL, high-affinity binding of HDL was calcium insensitive. After 4 hr incubation at 37°C, about 75% of the cell-associated HDL was released by trypsin, indicating that HDL binding is largely confined to the cell surface with minimal or no internalization of the lipoprotein. Virtually no degradation of HDL was observed when binding was performed at 0°C followed by transfer of the cells to 37°C. In that experiment, most of the bound HDL was

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released back to the medium within 2 hr at 37°C, indicating that binding (at 0°C) is reversible. This release, however, was temperature-sensitive and did not occur at 0°C. More recently, up-regulation of HDL binding was observed when the cellular cholesterol content was increased by pre-incubation with either cholesterol or LDL (354). The up-regulation process was proportional to the free cholesterol content of the cells and was inhibited by cycloheximide. Binding of HDL_3 could be increased 4- to 5-fold, and this up-regulation process could be down-regulated when cholesterol efflux preceded the binding assay. As for original cells, most of the bound HDL was trypsin-releasable and no, or only minimal, degradation was observed. Up-regulation of HDL binding by cholesterol enrichment was also reported for cultured endothelial cells (355). Analysis of the apoprotein content of different HDL preparations indicated that binding is proportional to their apoA-I content, but not apoA-II (353). Yet, in a more recent report, both apoA-I and apoA-II (as well as apoC-III) proteoliposomes compete with HDL_3 binding to fibroblasts (356). Of particular interest is the observation that blockage of tyrosine residues (but not lysine or arginine residues) nearly abolishes binding.

The potential biological significance of these observations was investigated by studies on cellular cholesterol metabolism. In spite of similar binding of the different HDL preparations, distinctly different effects were observed for HDL_2 as compared to HDL_3 or VHDL. HDL_3 or VHDL enhanced rates of sterol synthesis, inhibited cholesterol esterification, reduced cell cholesterol content, and increased LDL receptor activity and LDL degradation (357). Incubations with HDL_2 had opposite effects. When HDL_2, moreover, was added to HDL_3 or VHDL, the HDL_2 "blocked" the effects of the other HDL's (358). In that experiment, HDL_3 and VHDL were shown to promote sterol efflux from the cells. These observations strongly support the hypothesis that specific HDL binding sites facilitate cholesterol efflux from cells, and that this activity is related to the type of HDL used, HDL_2 or denser HDL populations.

C. Cholesterol efflux and reverse cholesterol transport

Movement of free cholesterol molecules to and from HDL has been mentioned in various sections of this review. The term "reverse cholesterol transport" is reserved for that part of the cholesterol flux that describes net movement of free cholesterol molecules out of cells. This cholesterol binds to components in plasma and eventually is used by other tissues, predominantly the liver. HDL appears to play a central role in the reverse cholesterol transport system.

Flux of cholesterol through cells in culture was studied extensively in the 1960's. Much of this research was summarized by Rothblat in 1969 (359). While summarizing his own studies and those of others (mainly Bailey's experiments), Rothblat suggested the following scheme for cholesterol metabolism in tissue culture cells. Cholesterol enters cells from lipoproteins by two mechanisms. The first is extraction of free cholesterol from lipoproteins, leaving behind particles poor in free cholesterol. This occurs through a physical process and is not mediated by cellular enzymes. The second is uptake of cholesteryl esters, presumably as part of the lipoprotein particle. Cell cholesterol content, however, is kept unchanged because of excretion of surplus free cholesterol from the cells, when an appropriate acceptor is present in the medium. The scheme suggested that HDL plays a major role in the excretion process, a suggestion that was subsequently verified (360).

Surprisingly, very little has been added to these concepts during the past 15 years. Apparently, all lipoproteins, including HDL, can donate free cholesterol to cells under appropriate conditions. For that to occur, the free cholesterol to phospholipid molar ratio in the lipoproteins should exceed that in the cell membrane. As discussed in Section V, that occurs infrequently because of the rapid consumption of lipoprotein free cholesterol by the LCAT reaction. Yet, tissues that use cholesterol for metabolic purposes (e.g., liver, adrenals, and gonads) may use this pathway to extract free cholesterol from lipoproteins. We have investigated this reaction in mycoplasma species and mycoplasma membranes (361–363). Free cholesterol transfer from either LDL or HDL was documented. The transfer was dependent on the free cholesterol to phospholipid molar ratio in the membranes and was facilitated by a protease-sensitive binding site for lipoproteins (investigated for LDL only (363)). Uptake of free cholesterol by mammalian tissues with a low free cholesterol to phospholipid ratio can occur by a similar mechanism. Supply of cholesteryl esters to cells with lipoproteins (including HDL) has been described earlier in this section. Degradative events and preferential extraction of cholesteryl ester molecules are involved. Undoubtedly, in most tissues, LDL catabolism is the major source of cholesteryl esters.

The mechanisms involved with egress of cholesterol from cells have been investigated in depth. In Rothblat's experiments (359, 360, 364, 365), delipidized serum alone, delipidized serum supplemented with phospholipids, or albumin + phospholipids enhanced cholesterol excretion. Albumin itself had little capacity to accept cholesterol, and addition of phospholipids to delipidized serum doubled the amount of cholesterol efflux (365). Sphingomyelin and lecithin were equally effective and considerably superior to phosphatidylethanolamine, while

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rabbit serum (365) and later HDL (360, 366) were shown to be the best acceptors. In some of these experiments, cholesterol synthesis by the cells was assessed. Efflux of cholesterol caused increased cholesterol synthesis, while influx resulted in decreased synthesis (360, 366). Subsequent studies by several different investigators have corroborated the earlier observations. Stein et al. (367–373) have shown that HDL or apolipoprotein-phospholipid complexes induce cholesterol egress from a variety of cell types, including cells that were pre-loaded with cholesteryl esters (371–373). Cholesterol efflux was documented with several different apoproteins and different phospholipids (370). Lipoprotein-deficient serum (LPDS), phospholipid-albumin complexes, HDL, delipidized HDL, and even phospholipids alone or intact erythrocytes are all effective in inducing cholesterol efflux from cholesterol-enriched cells in culture (374–378). As expected, cholesterol efflux induces not only cholesterol synthesis but also LDL-receptor activity (325, 346, 357, 379, 380).

Insights into the mechanism of cholesterol efflux have been reported, while using phospholipid vesicles as acceptors (170, 381, 382). Efflux was shown to depend on desorption of free cholesterol molecules into the aqueous phase and, with cells, on diffusion of the molecules through unstirred water layers (381). Binding of the acceptor to the cell membrane would facilitate the latter process and enhance efflux. Binding of HDL to specific sites on cell membranes such as described by Oram et al. (354) and others (383) possibly provides an adequate explanation for the central role that HDL plays in the cholesterol efflux process. Yet, other components present in serum may be as effective as HDL (384). Interestingly, the total plasma efflux capacity decreases by 25–50% when plasma is filtered through immunoaffinity chromatography on an immobilized anti-albumin antibody column (385).

A role for LCAT in the reverse cholesterol transport process was suggested in 1968 (386). It has been assumed that utilization of free cholesterol by LCAT allows movement of molecules from cell membranes to HDL. Experiments to test the contribution of LCAT (and hence of the lipid transfer proteins) to cholesterol efflux and reverse cholesterol transport were carried out during the last few years by Fielding et al. (385, 387–389). These investigators used immunoaffinity chromatography to separate lipoprotein populations and differentiated between efflux (rates of appearance of radioactive cholesterol in medium) and net mass transport of cholesterol from cells to medium. Removal from plasma of an apoA-I population unassociated with other apoproteins greatly reduced both efflux and net sterol transport (387). Removal or inhibition of LCAT did not change efflux but reduced mass transport. Removal of apoB, apoE, and apoA-II (which includes about 95% of plasma apoA-I associated with the A-II) in contrast, had almost no effect on either efflux or mass transport. Removal of albumin reduced efflux, but was without effect on mass transport (385). The capacity of plasma from different patients to remove cholesterol from the cell was reported to vary widely. Reduced or even reverse capacity was found in subjects with hyperbetalipoproteinemia, dysbetalipoproteinemia, and hypertriglyceridemia associated with cardiovascular diseases (389), and in patients with poorly controlled noninsulin-dependent diabetes (390). Of interest, both efflux and net mass cholesterol transport were saturated at 1–2% plasma concentrations (385, 387, 388). On the basis of these observations, it has been suggested that efflux occurs by two plasma components: albumin and a specific apoA-I population. Efflux with albumin is fully reversible and therefore does not cause mass transport. Efflux with apoA-I, however, is coupled with cholesterol esterification in HDL and cholesteryl ester transport to VLDL and LDL, and therefore results in net mass cholesterol movement (388). Apparently only a small fraction of the plasma apoA-I that is unassociated with other apoproteins is active in this process, and with cultured cells only small amounts of plasma are required to saturate both efflux and mass transport.

D. HDL receptors and post-binding events

The data described above demonstrate the complexity of processes involved with the interactions of HDL with cells. Most tissues appear to possess binding sites for HDL that are discernible from either the B, E or the E receptors. These sites recognize a variety of proteins and lipoproteins, including LDL. The sites, therefore, appear to recognize a "primitive" protein or proteolipid sequence that possibly contains tyrosine residues. The specific binding of HDL to this site (or sites) is followed by several different post-binding events. The first is internalization and degradation of the HDL in lysosomes, similar in principle to the LDL degradation pathway. The second is supply of cholesteryl esters to the cells without appreciable degradation of the protein moiety of the HDL. The reaction appears to predominate in endocrine tissues, but also operates in the liver and possibly other cell types. Very close association between the HDL and the cells is needed to transfer cholesteryl ester molecules. Reversible fusion is possible. Because this process is regulated (e.g., in endocrine tissues), it probably involves binding sites that are sensitive to trophic hormones. The third post-binding event is followed by accelerated efflux and transport of free cholesterol from cells. The binding of HDL to cell mem-

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branes is possibly regulated by the amount of cholesterol in the cells; up-regulation is seen when the cells are enriched with cholesterol. LCAT apparently does not play a role in the binding process itself, but increases the capacity of the HDL to accept cholesterol from the cells, especially in the presence of lipid transfer proteins and of acceptors for cholesteryl ester molecules. The fourth and fifth events are transfers of free cholesterol or of apoproteins from the HDL to specific cells. These events were not studied in detail but are probably dependent on interactions of the HDL with specific binding sites. Finally, transfer of apoA-I alone (or with only a few lipid molecules) to cells may also occur.

It is difficult to propose a common denominator for all these events. It is yet possible that a single specific receptor is present in all cell types, but post-binding events differ between tissues. That may reflect a balance between physical and biological processes. Transfer of lipid molecules to and from cells may merely represent a physical phenomenon that qualitatively occurs in all tissues but quantitatively is dependent on the metabolic state of the cell. For example, in tissues that use cholesterol, these molecules will move from the HDL to the cell. In other cells, the movement of the molecules will be in the opposite direction. HDL degradation may also occur in all cell types, but quantitatively may differ between cells. Apparently liver and intestinal cells possess the highest degradative capacity, while fibroblasts and endocrine cells possess the lowest. Retroendocytosis of the HDL may predominate in the latter cell types; alternatively, such cells may possess an inefficient HDL internalization system. Many more experiments are obviously needed before the true nature of the HDL receptors can be evaluated.

IX. HIGH DENSITY LIPOPROTEINS AND ATHEROSCLEROSIS

Associations between low levels of HDL and the risk of developing atherosclerotic diseases, especially of the coronary arteries, have been demonstrated in numerous studies. The power of low HDL levels to predict clinical coronary events is probably as strong as that of high LDL levels (391). In most studies, however, total cholesterol, a component that represents only a small fraction of the HDL mass, was measured. HDL-cholesterol is inversely related to plasma triglyceride in subjects with normal (214, 392) or high triglyceride levels (215, 216). Although the cholesterol is linearly related to plasma apoA-I concentration, the correlation coefficient ranges between 0.6 and 0.7 (214). Yet, HDL-cholesterol estimates total HDL levels, and there is no reason to suspect that the negative association between HDL cholesterol and the risk of coronary disease will be substantially different from that of HDL mass. Re-discovery of this association in 1975 (1) has initiated much of the research discussed in the present review. Thus, it seems appropriate to consider at this point whether the data has indeed provided knowledge on the anti-atherogenic properties of HDL.

Two lines of thought have been developed to explain the negative association between HDL levels and arteriosclerosis. The first assumes that HDL itself, and in particular HDL_2, is actively involved in retarding the development of atheromatous lesions. The second assumes that high HDL levels, and again in particular high HDL_2, reflect a metabolic process that is anti-atherogenic. According to this second view, the HDL particles themselves do not provide protection and are not directly involved in any activity that is anti-atherogenic. The evidence for and against each line of speculation is discussed below.

Two mechanisms have been proposed to explain the anti-atherogenic activity of HDL (391). The first is inhibition of LDL uptake by cells of the artery wall and the second is facilitated reverse cholesterol transport from cells of the artery wall. The first mechanism is based on the observations that HDL competes with the binding and uptake of LDL in cultured cells (393, 394). This inhibition is observed with a great excess of HDL protein (HDL-LDL protein ratio of 2.5). Because degradation of LDL by the LDL-receptors is currently considered the best defence against atherosclerosis (395), it is difficult to envision how competition by HDL would be anti-atherogenic. The second mechanism, the HDL-dependent reverse cholesterol transport, is certainly important. The crucial issue is whether higher circulating HDL levels promote exit of cholesterol from cells significantly more than lower levels. The data described in Section VIII do not support and do not rule out this possibility. Most studies demonstrate the importance of HDL but do not indicate that higher levels are beneficial. In fact, HDL_3 seems to be the reactant lipoprotein rather than HDL_2 (357, 379, 396). As well, in humans with very low HDL levels (e.g., Tangier disease (397) or apoA-I Milano variant (398)), there is no evidence for accelerated arteriosclerosis. Interestingly, premature arteriosclerosis prevails when *HDL is totally absent or moderately reduced.* Total absence of HDL may severely impair fat transport in the lipoprotein system, while moderate reduction of HDL levels probably reflects "inefficient" fat transport. In these two situations, therefore, the accelerated arteriosclerosis may reflect the inefficient fat transport system in general and not the specific absence of HDL or reduced HDL levels. Thus,

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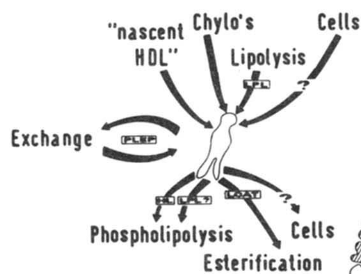

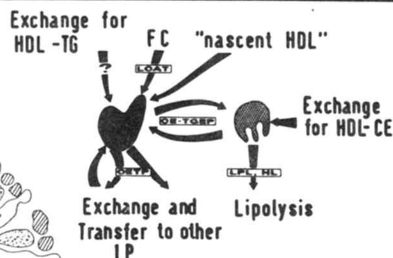

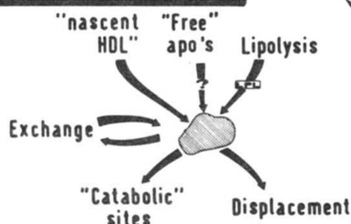

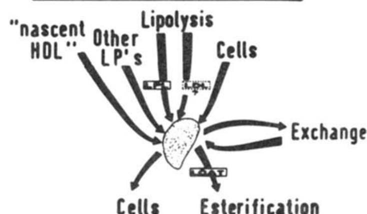

Fig. 11. The central position of HDL in the process of plasma fat transport system. The figure is a composite of pathways shown in Figs. 3–6.

while the HDL-dependent reverse cholesterol transport process is undoubtedly an important regulator of cell cholesterol homeostasis, the data are insufficient to relate this process to the negative association between HDL levels and arteriosclerosis.

Could high HDL levels reflect metabolic activities that are anti-atherogenic rather than be directly involved with prevention of atheroma formation? We believe that this possibility must be seriously considered. The data discussed in this review demonstrate the central position of HDL in plasma fat transport. This is illustrated in **Fig. 11** which summarizes the metabolic schemes shown in the previous figures. As is evident, almost all plasma events that regulate fat transport also affect HDL. Triglyceride transport in chylomicrons supplies HDL with A apoproteins and with phospholipids and free cholesterol. VLDL transport provides other apoproteins and very considerable amounts of surface lipids. Reciprocity is achieved by cholesterol esterification in HDL and cholesteryl ester transfer to lower density lipoproteins. Via these pathways, cholesterol leaves the plasma with LDL and "remnants," lipoproteins that interact with specific cell receptors. As mentioned above, such pathways for cholesterol exit from plasma are by their nature anti-atherogenic. When all plasma systems operate at high efficiency, HDL levels are expected to be high, HDL_2 will be formed, and its steady state levels also will be high. It is therefore suggested that high HDL levels reflect a basically "healthy" plasma fat transport system that does not cause atheroma formation. That suggestion must be rigorously tested.

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